AMIC-003-CMD

Evaluation of the AMICUS Red Blood Cell Exchange (RBCx) System in Sickle Cell Patients

Sponsor:
Fresenius Kabi USA LLC
Three Corporate Drive
Lake Zurich, Illinois USA 60047

Fenwal Inc. was acquired by Fresenius Kabi on December 1, 2012. Fenwal is a wholly owned subsidiary of Fresenius Kabi and is headquartered in Bad Homburg, Germany.

Confidential Information

I. SUMMARY OF CHANGES

A. VERSION 09 10FEB2017

Primary Sites and Primary Investigators		
Change		Reason for Change
a.	Added Information *Dr. Volker Witt* *St. Anna Kinderspital* *Kinderspitalgasse 6* *A 1090 Vienna*	Addition of clinical site.
Throughout Entire Document		
a.	Changed the total number of sites from 6 to 7.	The addition of St. Anna Kinderspital as a clinical site.
Appendix B		
a.	Addition of a label to be applied to FTX 1707, FTX 1708 and FTX 1709 for Non-US Clinical Sites.	To meet the additional requirements for Austria.
Appendix C		
a.	Addition of *Ethics Committee* to ICF language.	Addition of clinical site.

B. VERSION 08 05MAY2016

Throughout Entire Document		
Change		Reason for Change
a.	Changed the total number of enrolled subjects meeting inclusion and no exclusion criteria from 100 to 130.	To increase the total subject enrollment limit to ensure an adequate number of evaluable procedures.
Primary Sites and Primary Investigators		
b.	Deleted Nationwide Children's Hospital and added Phoenix Children's Hospital.	Nationwide Children's Hospital is not participating in the study; Phoenix Children's Hospital is a new site.

C. VERSION 07 08JAN2016

Throughout Entire Document	
Change	Reason for Change

a.	Changed the initial enrollment limit from 40 to 60.	To increase the initial adult subject enrollment limit.
Section 8.3. Waste Material Procedure		
a.	Replaced 10 mL sample requirement with *representative sample.*	To allow flexibility in the waste sample testing procedure while maintaining a representative sample draw requirement.
Appendix C SAMPLE INFORMED CONSENT AND ASSENT		
a.	Changed first person narrative to second person narrative.	To align with IRB procedures.

D. VERSION 06 17AUG2015

Throughout Entire Document		
	Change	Reason for Change
a.	Minor consistency/grammatical/spelling corrections	To correct typographical errors.
Participating Sites and Primary Investigators		
a.	Added information: *Washington University* *Brenda Grossman, MD, MPH* *660 S. Euclid Avenue, Box 8118* *Saint Louis, MO 63110* *Telephone: 314 362-6032* *Fax: 314 362-1461*	Addition of clinical site.
Section 6 SCOPE		
a.	Changed 5 to 6 sites	Addition of clinical site.
Section 8. STUDY PROCEDURES/METHODOLOGY		
a.	Removal of *automated* footnote from Table 5. SUMMARY OF REPLACEMENT RBC UNIT PARAMETERS	To allow spun hematocrit method to be performed.
Section 13.6. Evaluability Criteria		
a.	Removal of the evaluability criterion *If the procedure is terminated prior to delivering the target RF volume (±50 mL), then the FCR and End Hct are non-evaluable.*	To accommodate physician prescription.
Appendix C. Sample Informed Consent and Assent		
a.	Changed 5 to 6 sites	Addition of clinical site.

E. VERSION 05 08JAN2015

Throughout Entire Document

Change		Reason for Change
a.	Change of the statement *The supplement must be approved by the FDA prior to enrolling subjects six years old or older* to *The supplement must be approved by the FDA prior to enrolling pediatric subjects six – 17 years old*	To clarify continued enrollment of adult subjects during the interim analysis if the initial maximum enrollment of 40 adult subjects has not been met
Section 8. STUDY PROCEDURES/METHODOLOGY		
a.	Change of *type and cross match screening* language to *routine pre-procedure testing*	For clarity

F. VERSION 04 23DEC2014

Throughout Entire Document		
Change		Reason for Change
a.	Addition of an IDE supplement submission to the FDA following the DSMB review	To allow the FDA to review and approve the initial study data prior to enrolling subjects six years old or older
b.	Minor grammatical/spelling corrections	To correct errors
c.	Addition of an initial enrollment of up to 40 adult subjects	To limit enrollment to 40 adult subjects to assess safety prior to enrolling subjects six years old or older
Section 7. STUDY DESIGN		
a.	Removal of *medically stable subjects prescribed RBC Exchange or RBC Depletion/Exchange procedure(s) prior to surgery* inclusion criterion	To not exclude subjects that do not require pre-surgery treatment
b.	Addition of *prophylactic procedures that occur prior to surgery may be completed as part of the study* to the study design narrative	For clarity
Section 8. STUDY PROCEDURES/METHODOLOGY		
a.	Addition of *a pregnancy test will be performed on women with childbearing ability prior to the start of the procedure* to the subject evaluations narrative	For clarity
b.	Addition of type and cross match screening information	To clarify and document expected study methods
c.	Addition of Table 2. TYPE & CROSS MATCH SCREENING RESULTS	To clarify and document expected study methods
Section 13. STATISTICAL METHODS AND SAMPLE SIZE		
a.	Addition of citation added to sample size calculation	To cite source
Section 15. REFERENCES		
a.	Addition of reference number ten	To cite source

G. VERSION 03 20NOV2014

	Change	Reason for Change
Throughout Entire Document		
a.	Removal of all COBE Spectra information; all study procedures/design related to paired study removed; including change of primary objective and Section 13. STATISTICS	Change to study design
b.	Change of N from 30 paired procedures to a minimum of 62 single-arm procedures	Change to study design
c.	Removal of *RBC* from any reference to waste material	For accuracy; waste material also includes WBC, platelets, and plasma
d.	Minor grammatical/spelling corrections	To correct errors
Section 4. BACKGROUND		
a.	Modification to sub-section 4.2 Pre-Clinical Studies: information was updated and moved to Appendix D; addition of statement that study results are summarized in Appendix D	To present study results from the completed AMIC-002-CMD pre-clinical study
Section 5. OBJECTIVES		
a.	Change of secondary objective *Monitor subject safety through adverse events during the procedure and approximately 18-24 hours post-procedure, vital signs, laboratory parameters pre- and post-procedure, and unexpected adverse device effects* to *Evaluate device related serious adverse events during the procedure and approximately 18-24 hours post-procedure*	For clarification; the secondary objective is to determine the safety of the device; however, all AE/SAEs will be evaluated in the safety analysis
Section 6. SCOPE		
a.	Removal of requirement that approximately half of the procedures should be RBC Exchange and half should be RBC Depletion/Exchange	Procedures will follow standard of care at each site; therefore the contribution by each site for each procedure (RBC Exchange or RBC Depletion/Exchange) may vary.
b.	Modification to Table 1. SUBJECT ENROLLMENT	To reflect updated enrollment plan
Section 7. STUDY DESIGN		
a.	Modification to Figure 2. Evaluation of the AMICUS RBCx System	To reflect updated study design
b.	Addition of inclusion criterion *Medically stable subjects prescribed RBC Exchange or RBC Depletion/Exchange procedure(s) prior to surgery*	To not exclude this patient population

c.	Change of life expectancy fewer than 6 months exclusion to fewer than 30 days	To not exclude this patient population
d.	Addition of statement allowing subjects to contribute only one evaluable AMICUS procedure to the study	To reduce bias

H. VERSION 02 10NOV2014

Throughout Entire Document		
Change		Reason for Change
a.	Removal of Red Blood Cell (RBC) Depletion information; subsequent minor modifications to text to reflect the removal of the RBC Depletion procedure from study design	RBC Depletion procedures will not be performed in the United States (US)
b.	Removal of French regulations, site, investigator, and study design information	Investigation is limited to the US
c.	Modification to the primary objective	To consistently define the comparator as being the *mean difference* of the target to actual fraction of cells remaining (FCR) in the Test device to the *mean difference* of the target to actual FCR in the Control device
d.	Addition of *sickle cell disease/sickle cell*	To specify the intended treatment group
e.	Decrease of participating investigators from *up to 6* to *up to 5*; decrease of participating sites from *up to 6* to *up to 5*	Investigation is limited to 5 US institutions
f.	Change of *Gender* to *Sex*	Consistent with FDA guidance
g.	Addition of adverse event assessment approximately 18-24 hours post-procedure	To require post-procedure adverse event assessment
h.	Modifications to the in-text citations	To match updated Section 15. REFERENCES
i.	Minor spelling and grammatical changes; minor additions and rewording to the previous text to expand on the original thought being conveyed	For clarification and to correct errors
Title Page		
a.	Addition of *Sickle Cell Patients* to the study title	The investigation is limited to subjects with sickle cell disease
b.	Removal of European Community Representative	Investigation is limited to the US
Section 3. ABBREVIATIONS		
a.	Addition of *Hb C: Hemoglobin C* to list of abbreviations	To define the *Hb C* abbreviation

b.	Addition of *Hb F: Hemoglobin F* to list of abbreviations	To define the *Hb F* to list of abbreviations
c.	Addition of *RID: Rapid Infusion Device* to list of abbreviations	To define the *RID* abbreviation
d.	Modification to *SDV*: change of *Source Document Verification* to *Source Data Verification*	To correct error
Section 4. BACKGROUND		
a.	Removal of *abnormal, excess,* and *due to overproduction* when the words pertain to blood removal	Instructions For Use (IFU) has been modified to remove broad indications
b.	Removal of information about protozoan infections, carbon monoxide poisoning, thalassemia, and other pathologies of circulating RBCs	To be specific to the indicated subject population
c.	Change of *prevent secondary strokes* to *reduce secondary strokes*.	To eliminate the implication of treatment efficacy
d.	Addition of concurrent RBCs 510k number *BK000039*	For clarification
e.	Modification to a prior non-clinical study objective; addition of *obtained from patient procedures to evaluate the effects of abnormal RBCs when using the AMICUS RBCx System*	To expound and clarify
f.	Additions to the description of the *in* vivo study being completed in the Lake Zurich Donor Room in subsection 4.2. Pre-Clinical Studies	To expound and clarify
Section 6. SCOPE		
a.	Inclusion of an enrollment plan that explains the intent to enroll and summarize safety data from adult subjects (N=~15) prior to enrolling all eligible subjects (N=~15); safety data will be reviewed by an independent Data Safety Monitoring Board (DSMB)	To clarify the Sponsor's enrollment procedure and rationale
b.	Modifications to Table 1. SUBJECT ENROLLMENT; addition of *Age* column and *interim report* row; updates to *N* column; modifications to the footnotes	To depict the updated enrollment plan
c.	Modification to the statement *Data collected from this study will be summarized and submitted to TÜV and the FDA;* change to *Data collected will be summarized and*	To clarify the Sponsor's submission intentions

	submitted to the Fresenius Kabi European notified body, Technischer Überwachungs-Verein (TÜV) in Germany. Data from the completed study will be submitted to the United States Food and Drug Administration (FDA) to support a premarket submission	
Section 7. STUDY DESIGN		
a.	Modification to Figure 2. Evaluation of the AMICUS RBCx System; updates to the study population description	To depict the updated study design
b.	Addition of subsection 7.1.1. Lead-In Phase	To allow each site to perform approximately two, non-randomized, non-paired RBCx procedures on the AMICUS Separator for training purposes
c.	Modification to the inclusion criteria to specify subjects must have *documented diagnosis of a type of sickle cell disorder*	Investigation is limited to patients with sickle cell disease
d.	Removal of weight requirement for study participation from subject inclusion criteria	Subject inclusion will not be dependent upon the subject's weight
c.	Addition of *Subjects with sufficient vascular access to accommodate the RBCx procedure as determined by the medical staff responsible for obtaining venous access* inclusion criterion	To ensure subject has adequate vascular access to perform the RBCx procedure
d.	Addition of *Subjects who are able and agree to report adverse events (AEs) during the required reporting period* inclusion criterion	To ensure subject is able and willing to convey all adverse events during the procedure and via telephone approximately 18-24 hours post-procedure
e.	Modification to the *adequate availability of leukoreduced compatible replacement RBC products* inclusion criterion to include sickle trait negative, ABO blood group, Rhesus factor D (Rh (D) and non-expired standards	To specify minimum replacement fluid critieria
f.	Modification to the inclusion criterion *Medically stable subjects with a physician's prescription for RBC Exchange or RBC Depletion/Exchange treatment;* change to *Medically stable subjects who have been previously treated for sickle cell disease with RBC Exchange or RBC Depletion/Exchange*	To establish that first study procedure is not the subject's first exposure to RBCx treatment
g.	Addition of the exclusion criterion *In the*	To ensure subjects will be able to complete

	opinion of the investigator, subjects who have a life expectancy fewer than 6 months from time of first procedure	paired procedures
h.	Addition of the exclusion criterion *subjects who refuse blood products*	To exclude subjects who would not consent to receive replacement blood products
i.	Modification to the exclusion criterion *Subjects who experience a serious adverse event with the first RBC Exchange or RBC Depletion/Exchange;* change to *Subjects who have experienced a serious adverse event associated with an RBCx procedure in the past*	To exclude subjects who have previously had an adverse event during an RBCx procedure
j.	Removal of exclusion criterion *Subjects who have been treated with experimental anti-sickling medication/treatment (except hydroxyurea) within 30 days of Study Visit 1*	To allow this population to participate in the investigation
k.	Modification to the exclusion criterion *History of drug abuse, alcohol, or other factors that in the opinion of the investigator could affect the ability of the subject to comply with the requirements of the protocol;* removal of *history*	To exclude only those subjects who currently have substance abuse problems that could interfere with study participation, not subjects who have had substance abuse problems in the past
l.	Addition of subsection 7.3. Subject Enrollment	To define subject enrollment and requirements
m.	Addition of subsection 7.3.1. Procedure Deferral Criteria	To define procedure deferral criteria and requirements
n.	Addition of subsection 7.4. Screen Failures	To define screen failures and requirements
Section 8. STUDY PROCEDURES/METHODOLOGY		
a.	Addition of language to define location of testing and when they will be performed; complete blood count and hemoglobin profile will be evaluated locally according to institutional SOPs; plasma hemoglobin will be evaluated centrally at one laboratory	To clarify the standardization plan for study testing
b.	Addition of *The blood warmer cannot be used as a rapid infusion device (RID)*	To explain that if a blood warmer is used, it cannot be used as a RID
c.	Addition of language defining the type(s) of anticoagulant used; to explain that in addition to anticoagulant citrate dextrose, Formula A (ACD-A), custom anticoagulant may be used and should be defined by the prescribing physician based on the subject's medical need	To clarify the Sponsor's plan to standardize anticoagulant use across sites

d.	Modification to medication evaluation requirements; in addition to collecting medications that are taken on the day of procedure, site will collect concomitant medications taken up to 7 days prior to the procedure and during	For safety assessment
e.	Addition of language *a day-of-procedure CBC must be obtained*	To emphasize the requirement of a day-of-procedure study CBC to be obtained; regardless of the standard clinical tests performed, a day-of-procedure study CBC must be obtained to assess procedure evaluability
f.	Addition of footnote to *Post-procedure* column in Table 2. SUMMARY OF SUBJECT EVALUATIONS to require post-procedure sample to be taken prior to reinfusion, if performed, and within 10-15 minutes after the procedure if reinfusion is not performed	To ensure consistency across sites and treatment data collection processes
g.	Addition of *Age* to the required subject evaluations; addition of age to Table 2. SUMMARY OF SUBJECT EVALUTIONS; age at signing of informed consent will be reported	To ensure subject is eligible to participate; to ensure appropriate informed consent processes are performed
h.	Addition of *pre-procedure samples should be drawn* after kit installation	To clarify study procedure process
i.	Addition of *A sample for testing of each RF RBC unit should be taken prior to attachment of the unit to the kit*	To clarify study procedure process
j.	Addition of *Post-procedure samples must be drawn from the inlet line*	To clarify study procedure process
k.	Removal of the requirement *10 milliliters of blood should be drawn and discarded as waste prior to taking the post-procedure sample*	For clarification; requirement no longer necessary since blood is being drawn from the inlet line (see Summary of Changes comment *j* in *Section 8. STUDY PROCEDURES/METHODOLOGY*)
l.	Addition of *Automated* footnote to Tables 2, 4, and 5	To clarify that the *Hematocrit* value obtained is from the automated CBC report
m.	Addition of *End Time (Procedure Completed)* to Table 3. SUMMARY OF PROCEDURE EVALUATIONS	To document the end of procedure for AE assessment purposes
n.	Modification to waste material procedure description	For clarification
o.	Removal of *Primary Waste Container* and *Secondary Waste Container*	For clarification

p.	Addition of *Type of RBC Unit, ABO Type, Date Sample Drawn, Confirmation of Sickle Trait Negative, Confirmation of Leukoreduction, Confirmation of Rh (D) Compatibility, Expiration Date* to Table 5. SUMMARY OF REPLACEMENT RBC UNIT PARAMETERS	To ensure replacement RBC unit meets the criteria for use
Section 9. DESCRIPTION OF DEVICE		
a.	Modification to the intended use to a broad intended; statement that the protocol is limited to Red Blood Cell Exchange and Red Blood Cell Depletion/Exchange procedures	To explain the recognized intended use of the AMICUS Separator; the investigation is limited to sickle cell disease
b.	Addition of subsection 9.4. Indications for Use	To notify investigators of the RBCx System's indications for use
c.	Addition of subsection 9.5. Contraindications for Use	To notify investigators of the RBCx System's contraindications for use
d.	Addition of the AMICUS Operator's Manual, Volume 5, RBCx (REC-009200) to subsection 9.6.1. Investigational Materials	To document the investigational materials used
e.	Addition of the AMICUS Operator's Manual, Volume 1, Operation Basics, (07-19-07-291) or equivalent to subsection 9.6.2. FDA Cleared Materials	To document the FDA cleared materials used
f.	Addition of *custom anticoagulant may be used and should be defined by the prescribing physician based on the subject's medical need* to subsection 9.6.2. FDA Cleared Materials	To clarify the Sponsor's plan on how to standardize anticoagulant use across sites
Section 10. RISK ANALYSIS		
a.	Modification of the risk analysis to include and expound on information about the risks associated with apheresis, transfusion, hemolysis, air embolism, blood clotting, and fluid imbalance	For clarification; to document the possible risks and how the risks are mitigated
b.	Addition of subsection 10.2. Risks to Pediatric Subjects	To document the possible risks to children; to document the risks that are more common in children and how to risks are mitigated
c.	Change of *hemoglobinopathies* to *sickle cell disease*	The investigation is limited to sickle cell disease
Section 11. SAFETY EVALUATIONS		
a.	Modification of the Adverse Event Grading Criteria	To be consistent with the FDA's New Draft Guidance on Safety Data Collection

b.	Modification to *Relationship to Study Article* to *Relationship to Study Device*	To grade an adverse event in relation to the device; to differentiate the adverse event related to the device from an event related to the procedure
c.	Addition of Relationship to Procedure section	To grade an adverse event in relation to the event; to differentiate the adverse event related to the procedure from an event related to the device
d.	Modification to the definitions of the Adverse Event Relationship Criteria	To be consistent with FDA's New Draft Guidance on Safety Data Collection
Section 12. Data Quality Assurance		
a.	Addition of subsection 12.1. Data Reporting	To define data reporting and requirements
b.	Clarification and description of the monitoring procedures that will be performed	To ensure the protection of the rights, safety, and welfare of the subjects involved in the clinical investigation and the integrity of the resulting data
Section 13. STATISTICAL METHODS AND SAMPLE SIZE		
a.	Addition of the overall study success criteria	To define the Sponsor's plan for determining study success
b.	Additions to subsection 13.2. Determination of Sample Size to expound on sample size determination description	For clarification
c.	Additions to subsection 13.3. Analysis Data Sets to expound on the analysis data sets and criteria plan description, including plan for intent to treat population	For clarification
d.	Addition to subsection 13.3.1. Safety Analysis to expound on adverse event, safety, and system performance assessment description	For clarification
e.	Addition of *non-inferiority hypothesis for the mean of the paired effect* formula; addition of non-inferiority description	For clarification
f.	Addition of description of analysis of covariance (ANCOVA) use	For clarification
g.	Addition of the evaluability criterion *If the subject hematocrit input value and the actual day of treatment pre-procedure hematocrit differs by more than five (5) hematocrit points, then data for FCR and END Hct are non-evaluable*	To clarify evaluability criteria

h.	Additions to the interim analysis report description	To define the Sponsor's plan of when the Interim Analysis will be completed, what information will be included, by whom it will be reviewed, and the purpose for completing the analysis
Section 14. ADMINISTRATIVE RULES		
a.	Addition of the requirement for age and time of consent to be documented on the informed consent form	To ensure subject's eligibility and appropriate informed consent processes are performed
b.	Addition of subsection 14.10. Publications	To define Sponsor's publication definitions and requirements
Section 15. REFERENCES		
a.	Modification to reference list	To remove articles that are no longer applicable to the protocol; to cite literature used in the protocol
Appendix		
a.	Appendix B. LABELS: Removal of Protocol ID and title from clinical inventory labels	Not required by US to include Protocol ID and title on clinical inventory labels
b.	Appendix D. SAMPLE INFORMED CONSENT AND ASSENT: Addition of *Assent* to title	Sample informed assent form was added to Appendix D
c.	Appendix D. SAMPLE INFORMED CONSENT AND ASSENT: Incorporation of all applicable changes made to the protocol into the Adult, Parent, and Adolescent Assent form	To provide the investigator with an accurate sample informed consent/assent form that includes the required elements of the protocol

Person(s) authorized to sign the protocol and the protocol amendment(s) for the Sponsor:

Carrie Pineda, Sr. Director
Applied Product Testing

Jason Friedmann, Senior Clinical Research Specialist
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Participating Sites and Primary Investigators

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1 PROTOCOL SUMMARY

Name of Sponsor: Fresenius Kabi USA LLC	**Name of Experimental Article:** AMICUS Separator 4R4580 with SW Version 5.0	**IDE Number:** G140172

Study Number/Title:
AMIC-003-CMD Evaluation of the AMICUS Red Blood Cell Exchange (RBCx) System in Sickle Cell Patients

Estimated Duration: 2.5 Years

Number of Subjects: Up to 130 enrolled

Number of Procedures:

RBC Exchange and RBC Depletion/Exchange Procedures: A minimum of 62 evaluable procedures

Background:

The Sponsor has developed a new operating protocol on the AMICUS Separator that enables the device to perform Red Blood Cell Exchange (RBCx) procedures in accordance with the replacement fluid(s) (RF) prescribed by a physician for patients with sickle cell disease (SCD). The goal of these procedures is to remove a patient's red blood cells (RBCs) and replace the blood volume removed with either healthy donor RBCs and/or colloid/crystalloid solutions. Depending on the RF used, the procedure can be considered an RBC Exchange or RBC Depletion/Exchange procedure. The RBC Depletion/Exchange procedure is a modification of the RBC Exchange procedure. The AMICUS RBCx protocol provides the ability to use various RFs including healthy donor RBCs or a combination of fluids, such as saline followed by RBCs. In RBC Exchange and the exchange portion of the RBC Depletion/Exchange procedures, RBC units are used as the RF, while colloid and/or crystalloid solutions are used as the RF in the depletion portion of the RBC Depletion/Exchange procedures.

Objective:

Primary Objective:

To evaluate the accuracy of the subject's original RBCs remaining (Actual FCR) as measured by subject's post-procedure Hb S at the end of the procedure to the Target FCR

Secondary Objectives:

- To evaluate the accuracy of subject hematocrit post-procedure (End Hematocrit)*
- To evaluate subject cellular loss (white blood cells, platelets) post-procedure
- To evaluate device related serious adverse events during the procedure and approximately 18-24 hours post-procedure
- To evaluate the cellular content of the waste material

*For RBC Depletion/Exchange procedures, End Hematocrit will be measured at the end of the depletion phase and at end of the exchange phase.

Study Design:

This study will be performed under the direction of up to seven investigators at up to seven sites in the United States (US) and Europe. Data collected will be summarized and submitted to the Fresenius Kabi European notified body, Technischer Überwachungs-Verein (TÜV), in Germany. Data from the completed study will be submitted to the United States Food and Drug Administration (FDA) to support a premarket submission.

This protocol will be a multi-center, single-arm, open label study to complete a minimum of 62 evaluable RBCx procedures. The type of procedure performed will be determined by the physician. Up to a total of 130 subjects meeting all eligibility criteria will be enrolled. Initially, up to 60 subjects 18 years old or older will be enrolled to complete approximately 31 evaluable procedures. Aft Up to seven US investigational sites will participate in this study. er the initial procedures are complete, an interim report including summary statistics and safety data will be reviewed independently by a Data Safety Monitoring Board (DSMB). Following review by the DSMB, the interim report will be submitted to the FDA for review as an IDE supplement. The supplement must be approved by the FDA prior to enrolling pediatric subjects six to 17 years old.

The AMICUS RBC Exchange and RBC Depletion/Exchange procedures will be evaluated using a multi-center, single-arm, open label study design in subjects with SCD. Each subject will complete one procedure during one Study Visit. Dual venous access will be required using the method prescribed by the physician (e.g., peripheral venipuncture, catheter, Central Venous Access Device [CVAD], etc.). The appropriate RF and procedure parameters as prescribed by a physician will be used. Subject vital signs will be taken and laboratory samples will be drawn pre- and post-procedure. Adverse events will be monitored during the procedure and via telephone approximately 18-24 hours post-procedure. In addition to pre- and post-procedure vital signs and laboratory samples, subjects participating in the RBC Depletion/Exchange procedure evaluation will also have vital signs taken and laboratory samples drawn post-depletion phase.

Inclusion/Exclusion Criteria:

Inclusion:

- Subjects ≥6 years old.
- Subjects with documented diagnosis of a type of sickle cell disorder who require RBC Exchange or RBC Depletion/Exchange treatment.
- Medically stable subjects who have been previously treated for sickle cell disease with RBC Exchange or RBC Depletion/Exchange.
- Subjects, or subject's legal representative, who have provided signed informed consent, and assent when applicable, prior to participation.
- Adequate availability of sickle trait negative, leukoreduced, ABO blood group, Rhesus factor D (Rh (D)) compatible, unexpired replacement RBC products.
- Subjects with sufficient vascular access to accommodate the RBCx procedure as determined by the medical staff responsible for obtaining intravenous access.

- Subjects who are able and agree to report adverse events (AEs) during the required reporting period.

Exclusion:

- Procedures that occur during acute hospitalization.
- Procedures prescribed within one week of discharge of a hospitalization.
- Subjects with altered mental status that would prohibit the giving and understanding of informed consent, and assent when applicable, who do not have a legally authorized representative.
- Drug abuse, alcohol abuse, or other factors that in the opinion of the investigator could affect the ability of the subject to comply with the requirements of the protocol.
- Subjects who have experienced a serious adverse event associated with an RBCx procedure in the past.
- In the opinion of the investigator, subjects who have a life expectancy fewer than 30 days.
- Subjects who refuse blood products.
- Subjects who are pregnant.
- Subjects who fail to comply with site requirements for cessation of medication that interfere or increase procedure risk.

Statistical Methods:

The RBC Exchange and RBC Depletion/Exchange procedures have the same therapeutic endpoint, FCR. Hypothesis testing will be performed to evaluate the primary objective of the 95% confidence interval around the mean ratio of actual to target FCR as measured by Hemoglobin S in the subjects pre- and post-procedure to a pre-defined FCR range of 0.75 to 1.25.

Hypothesis testing is not planned for secondary variables. Summary statistics (mean, standard deviation, median, minimum, maximum and count) will be presented for continuous parameters with their 95% confidence limits as applicable. Discrete parameters will be presented by total number and percentage. A detailed statistical procedure will be outlined in the Statistical Analysis Plan.

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LIST OF TABLES

LIST OF FIGURES

TABLE OF APPENDICES

3 ABBREVIATIONS

AABB	Formerly American Association of Blood Banks
ACD-A	Anticoagulant Citrate Dextrose-Formula A
ADE	Adverse Device Effect
AE	Adverse Event
APT DM	Applied Product Testing Data Management
APT SM	Applied Product Testing Site Management
APT Stats	Applied Product Testing Statistics
°C	Degrees Centigrade
CAP	College of American Pathologists
CE	European Conformity
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Act
CRF	Case Report Form
CRA	Clinical Research Associate
CV	Curriculum Vitae
CVA	Central Venous Access
CVAD	Central Venous Access Device
DVD	Digital Video Disc
EC	Ethics Committee
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
°F	Degrees Fahrenheit
FDA	Food and Drug Administration
FWA	Federal Wide Assurance
g	Grams
g/dL	Grams per Deciliter
GCP	Good Clinical Practice
Hb	Hemoglobin
Hb A	Hemoglobin A
Hb C	Hemoglobin C
Hb F	Hemoglobin F
Hb S	Hemoglobin S
HBs Ag	Hepatitis B Antigen
Hct	Hematocrit
HIPAA	Health Information Portability and Accountability Act

ICH	International Conference on Harmonisation
IEC	International Electrotechnical Commission
ICF	Informed Consent Form
IRB	Institutional Review Board
ISO	International Organization for Standardization
IVH	Isovolemic Hemodilution
kg	Kilogram
L	Liter
min	Minute
mL	Milliliter
MNC	Mononuclear Cell
µL	Microliter
PN	Part Number
RBC	Red Blood Cell
RBCx	Red Blood Cell Exchange
RF	Replacement Fluid
RID	Rapid Infusion Device
SAE	Serious Adverse Event
SCD	Sickle Cell Disease
SD	Standard Deviation
SDV	Source Data Verification
SOP	Standard Operating Procedure
SW	Software
TPE	Therapeutic Plasma Exchange
TÜV	Technischer Überwachungs-Verein (Technical Inspection Association)
UADE	Unanticipated Adverse Device Effect
US	United States
WB	Whole Blood
WBC	White Blood Cell

4 BACKGROUND

Red Blood Cell Exchange (RBCx) is a type of therapeutic procedure used to remove red blood cells (RBCs) from sickle cell patients, exchanging them for healthy donor RBCs and/or crystalloid or colloid solutions to maintain fluid balance. Depending upon the replacement fluid(s) (RF) used, the procedure can be considered an RBC Exchange or an RBC Depletion/Exchange procedure. The RBC Depletion/Exchange procedure is a modification of the RBC Exchange procedure. Removal of RBCs may be performed manually or using an apheresis device. The removal of RBCs and replacement with healthy donor RBCs is most frequently used in the treatment of patients with complications of sickle cell disease (SCD). Depending upon institutional practices, RBC Exchange may also be employed after an RBC depletion pre-step (also known as isovolemic hemodilution), in which the initial replacement fluid used is a crystalloid or colloid solution instead of RBCs (refer to Figure 1).

In SCD and its variants, patients have an abnormal hemoglobin gene(s). In the predominant homozygous form, sickle cell anemia, the hemoglobin S (Hb S) has a single amino acid mutation in the beta globin chain compared to normal hemoglobin A (Hb A). The deoxygenation of Hb S results in the formation of rod-like structures that stiffen the RBC into a crescent or sickle shape. The deformed RBCs have decreased flexibility and cause microvascular occlusions. This results in a cycle of cell sickling, blood flow stasis, and progressive hypoxia that leads to severe pain crises, stroke, ischemia, or infarction causing tissue damage. Reduction of the Hb S concentration to approximately 30% allows persistence of adequate levels of normal Hb A for several weeks and decreases the potential for clinical signs and symptoms.[1]

In some centers, an RBC Depletion/Exchange procedure is used to reduce secondary strokes in SCD patients. The RBC Depletion/Exchange treatment is a two-step process – an RBC depletion step is performed prior to the start of the RBC exchange portion of the treatment.[1] This additional pre-step has demonstrated several advantages including increased efficiency of Hb S removal, increased treatment intervals by two weeks, decreased the total number of procedures per patient over time, reduced RBC utilization by 33%, and lowered donor exposure and the risk for alloimmunization, as well as significantly decreased costs.[2]

The AMICUS Separator System is an automated blood cell separator indicated for the collection of blood components and mononuclear cells (MNCs). It has been marketed since 1997 for the collection of platelets and concurrent plasma using ACD-A anticoagulant (BK960005, BK990009). In 2002, cleared collection protocols were expanded to include concurrent RBCs and MNCs (BK000039, BK000047). Additionally, in 2012, AMICUS was cleared to perform therapeutic plasma exchange (TPE) (K111702).

The Sponsor has developed a new operating protocol on the AMICUS Separator that enables the machine to perform RBCx procedures in accordance with the RF prescribed by a physician. The

Sponsor's documented product development process (including risk management) has been followed to evaluate the safety, effectiveness, and functionality of the AMICUS RBCx System.

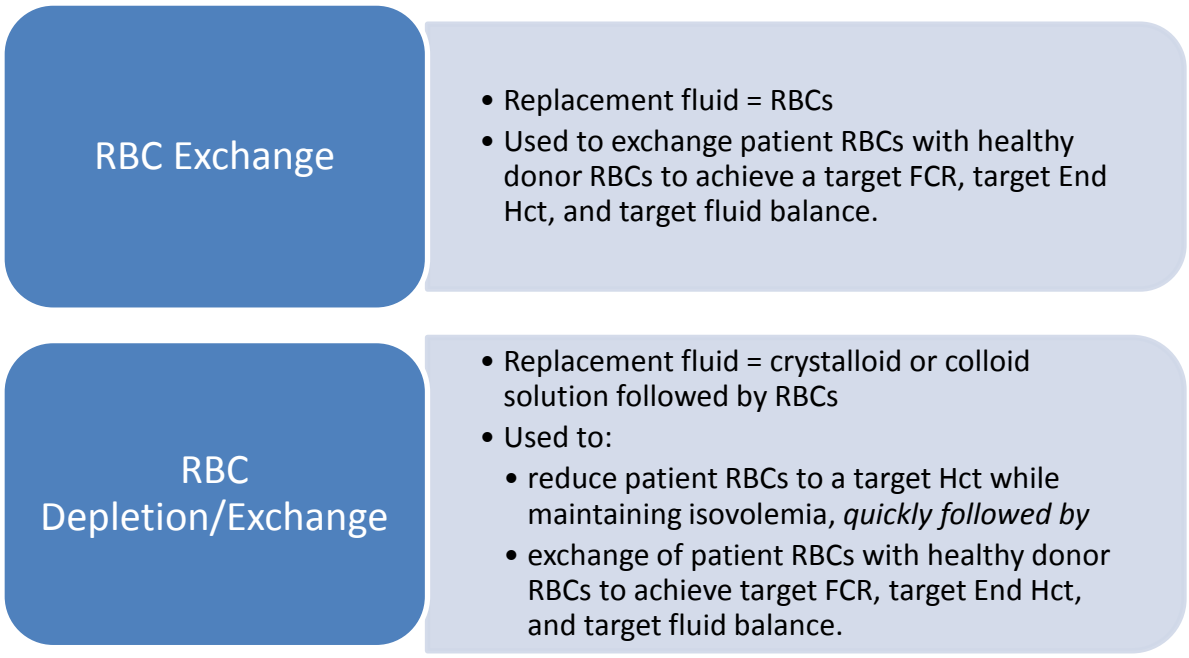

Figure 1. RBCx PROCEDURES

4.1 Prior Non-Clinical Studies

To ensure adequate subject protection, the development of the RBCx System included *in vitro* evaluations to assess performance, safety and efficacy, and completion of a Human Use Approval. A summary of the *in vitro* studies using bagged blood are found in Appendix D. The following objectives were evaluated:

- Confirm that RBC Exchange, RBC Depletion/Exchange and RBC Depletion procedures meet established internal requirements for key endpoints (e.g., FCR, End Hct and Fluid Balance accuracy).
- Evaluate the generation of hemolysis during procedures under worst case scenarios (high hematocrit, high hematocrit replacement fluid, high and low flow rates and small gauge needles).
- Perform RBC Exchange procedures using sickle cell waste blood obtained from patient procedures to evaluate effects of abnormal RBCs when using the AMICUS RBCx System. Also, the flow and pressure characteristics and hemolysis generation using high and low flow rates and small gauge needles was examined with these cells.

4.2 Pre-Clinical Studies

An *in vivo* study, AMIC-002-CMD, was completed in the Lake Zurich Donor Room. The results of the study are summarized in Appendix E.

5 OBJECTIVES

5.1 Primary Objective

To evaluate the accuracy of the subject's original RBCs remaining (Actual FCR) as measured by subject's post-procedure Hb S at the end of the procedure to the Target FCR.

5.2 Secondary Objectives

The following secondary objectives will be assessed:

- Evaluate the accuracy of subject hematocrit post-procedure (End Hematocrit)*
- Evaluate subject cellular loss (white blood cells, platelets) post-procedure
- Evaluate device related serious adverse events during the procedure and approximately 18-24 hours post-procedure
- Evaluate the cellular content of the waste material

*For RBC Depletion/Exchange procedures, End Hematocrit will be measured at the end of the depletion phase and at the end of the exchange phase.

6 SCOPE

It is estimated that a minimum of 62 evaluable RBC Exchange and RBC Depletion/Exchange procedures will be completed. Up to a total of 130 subjects meeting inclusion and no exclusion criteria will be enrolled. Initially, up to 60 subjects 18 years old or older will be enrolled to complete approximately 31 evaluable procedures. After the initial procedures are complete, an interim report including summary statistics and safety data will be reviewed independently by a Data Safety Monitoring Board (DSMB). Following review by the DSMB, the interim report will be submitted to the FDA for review as an IDE supplement. The supplement must be approved by the FDA prior to enrolling pediatric subjects six to 17 years old. The interim analysis will not evaluate the primary objective.

The scope is depicted in Table 1.

Table 1. SUBJECT ENROLLMENT

Age	RBCx System Procedure	N[1]
≥18 yrs	RBC Exchange	31
	RBC Depletion/Exchange	
	Interim Report Including Summary Statistics and Safety Data	
≥6 yrs	RBC Exchange	31
	RBC Depletion/Exchange	

[1]N = Approximate minimum number of subjects completing evaluable procedures

The evaluations of the RBC Exchange and RBC Depletion/Exchange procedures will be a multi-center, single-arm, open label study. The AMICUS RBCx procedures will evaluate the accuracy of the subject's original RBCs remaining (Actual FCR) as measured by subject's post-procedure Hb S at the end of the procedure to the Target FCR. Secondary objectives will be evaluated.

Subjects will be required to complete one study visit.

Up to seven investigational sites will participate in this study. All sites may enroll subjects for treatment with RBC Exchange or RBC Depletion/Exchange based on the physician's prescription.

Data collected will be summarized and submitted to the Fresenius Kabi European notified body, Technischer Überwachungs-Verein (TÜV), in Germany. Data from the completed study will be submitted to the United States Food and Drug Administration (FDA) to support a premarket submission.

7 STUDY DESIGN

The sites will perform this study in accordance with, but not limited to, the following applicable guidelines:

- The study protocol
- The World Medical Association Declaration of Helsinki (2013 Version)
- ICH E6 (R1) Consolidated Guideline for Good Clinical Practice (GCP)
- ISO 14155:2011 (E) Clinical investigation of medical devices for human subjects - Good Clinical Practice

- The privacy regulation of the Health Insurance Portability and Accountability Act of 1996
- 21 CFR 50 Protection of Human Subjects
- 21 CFR 54 Financial Disclosure by Clinical Investigators
- 21 CFR 56 Institutional Review Boards
- 21 CRF 640 Additional Standards for Human Blood and Blood Products
- 21 CFR 812 Investigational Device Exemptions

7.1 Overview of Study Design

This study will evaluate the AMICUS RBCx System in adult and pediatric subjects diagnosed with SCD. Subjects will complete one RBC Exchange or RBC Depletion/Exchange procedure on the AMICUS Separator at one study visit. The type of procedure performed (RBC Exchange or RBC Depletion/Exchange) will be as prescribed by the physician. Initially, up to 60 subjects 18 years old or older will be enrolled to complete approximately 31 procedures. After the initial procedures are complete, an interim report including summary statistics and safety data will be independently reviewed by a DSMB. Following review by the DSMB, the interim report will be submitted to the FDA for review as an IDE supplement. The supplement must be approved by the FDA prior to enrolling pediatric subjects six to 17 years old. The interim analysis will not evaluate the primary objective.

Procedures that occur during acute hospitalization will not be completed as part of the study. Procedures that are prescribed within a week of discharge from a hospitalization will not be completed as part of the study. Prophylactic procedures that occur prior to surgery may be completed as part of the study, provided the subject meets all eligibility requirements.

Target subject and procedure parameters will be entered at the beginning of the procedure and procedure summary data will be recorded. Adverse events, alarms, and device malfunctions that occur during the procedure will be recorded. Adverse events will be assessed approximately 18-24 hours post-procedure.

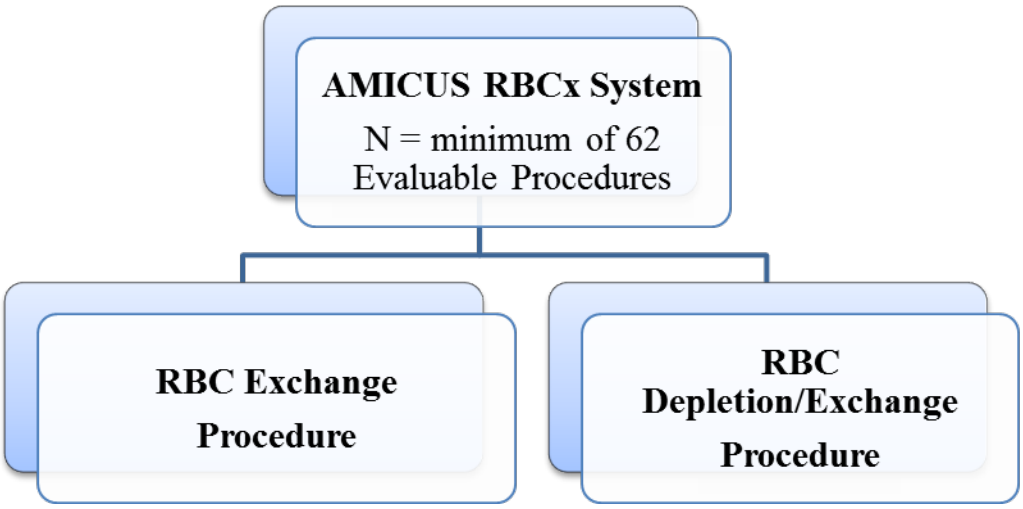

Figure 2. EVALUATION OF THE AMICUS RBCx SYSTEM

7.1.1 Lead-In Phase

Each site will be able to perform approximately two RBC Exchange or RBC Depletion/Exchange procedures on the AMICUS Separator for training purposes. Representatives of the Sponsor will be present for the lead-in procedures to provide training and support to the site regarding device set up, system performance, and procedure management. The lead-in phase subject population will include subjects 18 years old or older who provide informed consent and meet all eligibility criteria. The type of procedure performed, parameters, and RF will be as prescribed by the physician. Lead-in subjects will not be included in the evaluable population but will be included in the safety analysis.

Subjects participating in the lead-in phase may return to re-enroll into the full study if they meet eligibility criteria, provide new informed consent, and will be assigned a new Subject Identification number (Subject ID).

7.1.2 AMICUS RBC Exchange Procedure

The AMICUS RBC Exchange procedure will be evaluated using a multi-center, single-arm, open label study design in subjects with SCD. Dual venous access will be required using the method prescribed by the physician (e.g., peripheral venipuncture, catheter, Central Venous Access Device [CVAD]). The appropriate RF and procedure parameters as prescribed by a physician will be used. The subject's sickle cell diagnosis and demographics will be documented. Subject vital signs will be documented and laboratory samples will be drawn pre- and post-procedure. Adverse events will be monitored during the procedure and assessed approximately 18-24 hours post-procedure.

7.1.3 AMICUS RBC Depletion/Exchange Procedure

The AMICUS RBC Depletion/Exchange procedure will be evaluated using a multi-center, single-arm, open label study design in subjects with SCD. Dual venous access will be required using the method prescribed by the physician (e.g., peripheral venipuncture, catheter, CVAD). The appropriate RF and procedure parameters as prescribed by a physician will be used. The subject's sickle cell diagnosis and demographics will be documented. Subject vital signs will be documented and laboratory samples will be drawn pre-, post-depletion phase, and post-procedure. Adverse events will be monitored during the procedure and assessed approximately 18-24 hours post-procedure.

7.1.4 Waste Material

The waste material removed will be pooled, weighed, and assessed for cellular content. Refer to Section 8.3 for additional details.

7.2 Subject Selection

Subjects will be identified by the site investigator(s) who will discuss details of study participation. Each subject will provide written informed consent, and assent when applicable, as approved by the EC/IRB before participating in the study. If a subject is unable to provide written informed consent or assent, his/her legally authorized representative may sign on his or her behalf. All subject information will be kept confidential in compliance with all state and federal laws and regulations, including the HIPAA Privacy Rule. After choosing to participate, the subject can withdraw from the study at any time without prejudice, penalty or loss of benefits or care that he or she would otherwise be entitled. All subjects will be assessed for eligibility into the study using the inclusion and exclusion criteria.

7.2.1 Inclusion Criteria

- Subjects ≥6 years old.
- Subjects with documented diagnosis of a type of sickle cell disorder who require RBC Exchange or RBC Depletion/Exchange treatment.
- Medically stable subjects who have been previously treated for sickle cell disease with RBC Exchange or RBC Depletion/Exchange.
- Subjects, or subject's legal representative, who have provided signed informed consent, and assent when applicable, prior to participation.
- Adequate availability of sickle trait negative, leukoreduced, ABO blood group, Rhesus factor D (Rh (D)) compatible, unexpired replacement RBC products.
- Subjects with sufficient vascular access to accommodate the RBCx procedure as determined by the medical staff responsible for obtaining intravenous access.

- Subjects who are able and agree to report adverse events (AEs) during the required reporting period.

7.2.2 Exclusion Criteria

- Procedures that occur during acute hospitalization.
- Procedures prescribed within one week of discharge of a hospitalization.
- Subjects with altered mental status that would prohibit the giving and understanding of informed consent, and assent when applicable, who do not have a legally authorized representative.
- Drug abuse, alcohol abuse, or other factors that in the opinion of the investigator could affect the ability of the subject to comply with the requirements of the protocol.
- Subjects who have experienced a serious adverse event associated with an RBCx procedure in the past.
- In the opinion of the investigator, subjects who have a life expectancy fewer than 30 days.
- Subjects who refuse blood products.
- Subjects who are pregnant.
- Subjects who fail to comply with site requirements for cessation of medication that interfere or increase procedure risk.

7.3 Subject Enrollment

Enrollment will occur at the time of informed consent, and assent when applicable. After enrollment, the subject will be assigned a unique Subject ID. The procedure type will be as prescribed by the physician. Each subject may provide only one evaluable AMICUS procedure for the study.

7.3.1 Subject Re-enrollment

If a subject is able to establish vascular access and begin the procedure but does not complete the procedure, the procedure will not be evaluable (see Section 13.6 Evaluability Criteria). However, subjects not successfully completing the procedure may re-enroll into the study if they meet eligibility criteria, provide new informed consent, and assent when applicable, and will be assigned a new Subject ID. Subjects may re-enroll only one time into the study following an incomplete procedure.

7.4 Screen Failures

Enrolled subjects who do not meet all eligibility criteria will be considered a screen failure. The subject may then re-enroll into the study if they meet eligibility criteria, provide new informed

consent, and assent when applicable, and will be assigned a new Subject ID. Subjects may re-enroll only one time into the study following a screen failure.

7.5 Subject Drop-out

If the subject signs an informed consent form (ICF) but does not return to complete the study visit prior to the end of the study, the subject will be considered a drop-out.

8 STUDY PROCEDURES/METHODOLOGY

The AMICUS Separator will be operated by qualified healthcare professionals who are trained in the operation of the system. The Operator's Manual should be followed for RBCx procedures, kit installation, and instructions for general operation of the instrument.

Procedure parameters will be entered into the AMICUS Separator in compliance with the physician's prescription. Summary of routine pre-procedure testing data used for input calculations, subject evaluations, procedure evaluations, replacement RBC unit parameters, and waste material evaluations are listed in Tables 2, 3, 4, 5, and 6. The subject's complete blood count (white blood cell count (WBC), RBC, platelet count, and hematocrit; also known as CBC) and hemoglobin profile (Hb A, Hb S, Hb C, and Hb F) will be evaluated locally according to institutional SOPs. Plasma hemoglobin will be evaluated centrally at one laboratory.

If necessary, a blood/fluid warmer can be used according to the manufacturer's directions and following institutional SOPs. The return line should be connected to the blood warmer and primed prior to use. The blood warmer cannot be used as a rapid infusion device (RID).

Typically, anticoagulant citrate dextrose, Formula A (ACD-A) is used for therapeutic apheresis. Custom anticoagulant may be used and should be defined by the prescribing physician based on the subject's medical need.

A commercially available 10 L Waste Bag and Plasma Transfer Set will be provided and used to pool procedure waste material into one container to alleviate manual combining of multiple waste containers at the end of the procedure.

A sample of the hematocrit and plasma hemoglobin of each replacement RBC unit will be taken prior to transfusion and recorded. The replacement RBC unit parameters listed in Table 5 will be recorded. All units should be sickle cell trait negative, leukoreduced, ABO type and Rh (D) compatible, and meet institutional requirements for transfusion.

8.1 Subject Evaluations

For each subject enrolled, the date of birth, age, sex, height, weight, ethnicity, race, primary sickle cell diagnosis requiring RBCx, and concomitant medications taken up to seven (7) days prior to the procedure and during the procedure will be documented. A pregnancy test will be performed on women with childbearing ability prior to the start of the procedure. In addition, the following study evaluations will be assessed in each subject pre- and post-procedure: vital signs (blood pressure, temperature, pulse, and respirations), CBC, plasma hemoglobin, and a hemoglobin profile. Subjects requiring RBC Depletion/Exchange will have post-depletion phase vital signs taken and CBC and plasma hemoglobin samples drawn as well. Post-procedure and post-depletion phase sampling must be taken from the inlet line. Blood samples shall be collected in appropriate laboratory tubes per institutional SOPs. As needed, a three-way stopcock may be utilized to facilitate subject sampling.

Routine pre-procedure testing (e.g., type and cross-match, Hb S, and Hct) may be performed per institutional SOPs; however, a day-of-pre-procedure study CBC and Hb profile must be obtained. The Hb S and Hct values from the routine pre-procedure visit sample(s) may be used to calculate pre-procedure input values; once the day-of-procedure results are available, the Sponsor will compare the Hb S and Hct input values to the day-of-procedure Hb S and Hct values to establish procedure evaluability. Routine pre-procedure testing data used for pre-procedure input values are listed in Table 2; subject evaluations are listed in Table 3.

Pre- and post-procedure samples and waste material should utilize the same local methodology/equipment (e.g., cell counter, scale) for the samples. Plasma hemoglobin samples will be prepared, frozen, and shipped to a central laboratory following Sponsor requirements. Adverse events will be monitored during the procedure and assessed approximately 18-24 hours post-procedure.

Table 2. ROUTINE PRE-PROCEDURE TESTING DATA

Parameter	Pre-procedure
Actual Hemoglobin S value used for FCR calculation	X
Target Hemoglobin S value used for FCR calculation	X
Hematocrit value	X
Sample Date	X
Sample Time	X

Table 3. SUMMARY OF SUBJECT EVALUATIONS

Parameter	Pre-procedure	End of Depletion Phase[1]	Post-procedure[2]
Subject			
Primary Diagnosis	X	--	--
Date of Birth	X	--	--
Age[5]	X	--	--
Sex	X	--	--
Ethnicity	X	--	--
Race	X	--	--
Weight	X	--	--
Height	X	--	--
Medications taken day of procedure and up to 7 days prior	X	--	--
Vital Signs			
Blood Pressure	X	X	X
Temperature	X	X	X
Pulse	X	X	X
Respiration Rate	X	X	X
Laboratory			
White Blood Cell Count[3]	X	X	X
Red Blood Cell Count[3]	X	X	X
Platelet Count[3]	X	X	X
Hematocrit[3,7]	X	X	X
Plasma Hemoglobin[4]	X	X	X
Hemoglobin A[3]	X	--	X
Hemoglobin S[3]	X	--	X
Hemoglobin C[3]	X	--	X
Hemoglobin F[3]	X	--	X
Pregnancy Test[3]	X	--	--
Adverse Events	During the procedure and approximately 18-24 hours post-procedure[6]		

[1]Applicable for RBC Depletion/Exchange procedure
[2]If performing reinfusion, the operator must draw the post-procedure sample before reinfusion is performed. If reinfusion is not performed, the sample must be taken within approximately 10-15 minutes after the end of the procedure.
[3] Study Test performed locally according to institutional SOPs.

Parameter	Pre-procedure	End of Depletion Phase[1]	Post-procedure[2]

[4]Test performed at central laboratory.
[5]Age at signing of informed consent will be reported.
[6]Post-procedure assessment will be performed via telephone.
[7] Automated.

8.2 AMICUS RBCx Procedures

8.2.1 RBC Exchange Procedure

The operator will select RBC Exchange on the AMICUS display. A sample for testing each RF RBC unit should be taken prior to attaching to the kit (refer to Table 5). After the kit is installed, and primed, the subject and the RF units will be connected to the kit following institutional SOPs; the pre-procedure samples should be drawn and the procedure will start. Anticoagulated whole blood will be drawn through the inlet line and separated in the separation chamber of the centrifuge pack. RBCs, some plasma, WBCs, and platelets will be removed and transferred to the kit's waste container. The waste blood should be pooled in the 10 L Waste Bag. The plasma exiting the centrifuge will be combined with healthy donor RBCs and will be returned to the subject. The procedure will continue until the prescribed procedure targets are reached and the AMICUS will automatically stop.

The procedure may be ended with or without reinfusion. If reinfusion is desired, the operator must draw the post-procedure blood sample before reinfusion is performed or the procedure will be non-evaluable. If reinfusion is not performed, the blood sample must be taken from the subject within approximately 10-15 minutes after the end of the procedure. Post-procedure samples must be drawn from the inlet line.

After the procedure is complete, the subject will be disconnected and appropriate hemostasis care will be provided according to institutional SOPs.

The 10 L Waste Bag should be disconnected and aseptically sealed using the attached cap.

8.2.2 RBC Depletion/Exchange Procedure

The operator will select the RBC Depletion/Exchange procedure on the AMICUS display. A sample for testing each RF RBC unit should be taken prior to attaching to the kit (refer to Table 5). After the kit is installed and primed, the RF (i.e., crystalloid/colloid solutions) may be attached. The subject will be connected to the kit following institutional SOPs, the pre-procedure samples should be drawn, and the depletion phase will start. Anticoagulated whole blood will be drawn through the inlet line and separated in the separation chamber of the centrifuge pack. RBCs, some plasma, WBCs, and platelets will be removed and transferred to the kit's waste container. The waste blood should be pooled in the 10 L Waste Bag. The depletion phase will continue until the Target Depletion Hematocrit limit is reached. A post-

depletion blood sample from the subject must be taken prior to the start of the exchange phase. The post-depletion sample must be drawn from the inlet line. Once the Depletion phase is completed, the replacement fluid should be changed to the physician prescribed RF for the exchange phase. The procedure will continue with the exchange phase and will end once the procedure targets have been reached.

The procedure may be ended with or without reinfusion. If reinfusion is desired, the operator must draw the post-procedure blood sample before reinfusion is performed or the procedure will be non-evaluable. If reinfusion is not performed, the blood sample must be taken from the subject within approximately10-15 minutes after the end of the procedure. Post-procedure samples must be drawn from the inlet line.

After the procedure is complete, the subject will be disconnected and appropriate hemostasis care will be provided according to institutional SOPs.

The 10 L Waste Bag should be disconnected and aseptically sealed using the attached cap.

Table 4. SUMMARY OF PROCEDURE EVALUATIONS

Parameter Description	Pre-procedure Input/ Estimator Values	End of Depletion Phase Values[2]	End of Exchange Procedure Values
Procedure Performed (RBC Exchange, RBC Depletion/Exchange)	X	--	--
Venous Access Used	X	--	--
Replacement Fluid Used[2]	X	--	--
Total Blood Volume	X	--	--
Fraction Cells Remaining	X	--	X
Subject Hematocrit[1]	X	--	--
End Hematocrit[1]	X	--	X
Depletion Hematocrit[2]	X	X	--
Average Replacement Fluid Hematocrit	X	--	--
Replacement Volume to Subject	X	--	X
Fluid Balance	X	X	X
Maximum Whole Blood Flow Rate	X	--	X
AC Ratio	X	--	--
Whole Blood Flow Rate	X	--	X
Procedure Time	X	X	X
Anticoagulant to Subject	X	X	X
Anticoagulant Used	X	X	X
Other Replacement Fluid	X	--	--
Depletion Red Cell Volume[2]	X	--	--
Anticoagulant Type	X	--	--
Citrate Infusion Rate	X	--	X
Reinfusion	X		
Was prime diverted?	X	--	--
Custom Prime	X	--	--
Start Prime Hematocrit	X	--	--
End Prime Hematocrit	X	--	--
Whole Blood Processed	--	X	X
Plasma Returned[3]	--	X	--

Saline to Subject	--	X	X
Replacement Fluid Albumin Returned	--	X	X
Replacement Fluid RBC Returned	--	--	X
Replacement Fluid Saline Returned	--	X	X
Custom Replacement Fluid Returned	--	X	X
Was reinfusion performed?	--	--	X
End Time (Procedure Completed)	--	--	X
Device Alarms	During the Procedure		
Device Malfunctions	During the Procedure		

[1]For RBC Depletion/Exchange, the RBC Depletion subject hematocrits pre-procedure and at the completion of the depletion phase (Depletion Hematocrit) will be measured. The RBC Depletion Hematocrit may be used as the starting hematocrit for the RBC Exchange phase.

[2]Applicable for the RBC Depletion/Exchange Procedure.

[3]Plasma with anticoagulant. Applicable for RBC Depletion/Exchange Procedure.

Table 5. SUMMARY OF REPLACEMENT RBC UNIT PARAMETERS

Parameter	Pre-procedure
Type of RBC Unit[1]	X
ABO Type	X
Unit Identification Number	X
Date/ Time Sample Drawn	X
Hematocrit	X
Volume	X
Plasma Hemoglobin	X
Confirmation of Sickle Trait Negative	X
Confirmation of Leukoreduction	X
Confirmation of Rh (D) Compatibility	X
RBC Unit Expiration Date	X

[1]Additive Solution (AS) RBCs, CPD RBCs, CP2D RBCs, CPDA-1 RBCs, Washed RBCs, Other

8.3 Waste Material Procedure

Waste material should be in a single container prior to sampling. Pooling into a 10 L Waste Bag may be necessary. The bag containing the waste material should be weighed to calculate the volume removed. Care should be taken to ensure that the waste bag is folded and placed appropriately on the top loading scale to avoid inaccurate readings.

The waste material should be manually mixed well, taking care not to induce hemolysis. The sampling port on the 10 L Waste Bag should be flushed carefully prior to removing the sample to ensure a representative sample. A representative sample from the waste material should be drawn and used for testing. The remaining waste material and waste bag(s) should be discarded as biohazardous material according to institutional SOPs.

For RBC Depletion/Exchange procedures, the waste material from the depletion phase of the procedure should be collected separately from the waste material collected from the exchange phase of the procedure. A new container and a transfer set or comparable tubing set should be used.

Table 6. WASTE MATERIAL EVALUATIONS

Parameter	End of Depletion Phase[1]	Post-Exchange Phase
Gross Weight	X	X
Container Tare Weight	X	X
Hematocrit[2]	X	X
Platelet Count	X	X
White Blood Cell Count	X	X

[1]Applicable for RBC Depletion/Exchange procedure only.
[2]Automated

8.4 Appropriateness of Measures

Test measurements employed are standard in the industry or validated research methods.

8.5 Study Completion

The Sponsor considers the study complete after the last observation (last test completed).

8.6 Site Closure

The Sponsor considers the site closed after the collection of the final data/data clarification and closure of all queries, EC/IRB has been notified, and the Sponsor has completed the Site Closeout Visit.

8.7 Discontinuation

Details of discontinuation of subjects or the site may include, but are not limited to:

- Failure of the investigator to comply with the study protocol, regulatory requirements, Good Clinical Practices (GCP) guidelines or other study provisions.
- Safety concerns.
- Inadequate study subject enrollment by the investigator.

8.8 Study Termination

Site closure or study termination may be decided at any time by the Sponsor or site investigator, provided there is reasonable cause and/or sufficient notice is given in advance of the intended withdrawal, e.g., safety concerns.

9 DESCRIPTION OF DEVICE

9.1 AMICUS RBCx System

The AMICUS Separator is an FDA cleared (and CE marked) centrifuge-based apheresis system that will contain investigational software capable of performing a new RBCx operating protocol comprised of similar types of procedures based on the prescribed RF: RBC Exchange and RBC Depletion/Exchange. The AMICUS Separator is able to collect blood components of interest and return RF and the remaining blood components with saline and anticoagulant back to a patient. The new RBCx operating protocol is an extension of the intended use for the AMICUS Separator System. RBCx builds on processes that are already cleared for the AMICUS device system including RBC separation and collection, fluid return while maintaining fluid balance, and control and delivery of anticoagulant solution. The system already has proven capability in separating and collecting RBCs (in conjunction with a platelet collection, BK000039) with appropriate control and delivery of anticoagulant solution. The RBCx System is a continuous-flow process that utilizes the same hardware, technological characteristics and disposable kit (AMICUS Exchange Kit) as the cleared AMICUS TPE procedure (K111702). A new disposable waste transfer set has been developed to allow the transfer of removed RBCs from the smaller waste container on the front right scale hook to the two larger waste containers located on the side hook of the AMICUS Separator. An additional volume of the AMICUS Operator's Manual, Volume 5, RBCx (REC-009200) is available.

In general, whole blood is drawn from the patient, anticoagulated, and centrifuged in the AMICUS Separator. Separation occurs in the separation chamber of the centrifuge pack, while the collection chamber is filled with saline during prime and serves as balance for the separation chamber. The WBCs, RBCs, platelets, and plasma are separated in the kit's separation chamber.

The RBCs, some plasma, WBCs, and platelets are transferred to the waste containers. Anticoagulated plasma is mixed with the RF (healthy RBCs or albumin/saline) and pumped back to the patient.

During the procedure, the instrument uses weight scales and pumps to accurately track the RBC volume that has been removed from the patient and monitor the volume of RF or RBCs that have been returned. The AMICUS Separator uses algorithms to maintain appropriate hematocrit and fluid balance and achieve the desired volume exchange.

The RBCx software version is investigational; the AMICUS Separator containing the investigational software will be labeled in accordance with 21CFR 812.5. The AMICUS Exchange Kit and waste transfer set will also be labeled investigational.

A plastic overlay found on the top panel traces the replacement fluids, solutions, and blood paths and provides a visual aid during kit installation.

9.2 Intended Use

The AMICUS Separator, a blood component separator, is intended for use in therapeutic apheresis applications, and may be used to perform Red Blood Cell Exchange, Depletion, and Depletion/Exchange (RBCX) procedures.

This protocol is limited to the use of the RBC Exchange and RBC Depletion/Exchange procedures.

9.3 Indications for Use

The AMICUS Separator, a blood component separator, can be used to perform Red Blood Cell Exchange (RBCx) procedures for the transfusion management of Sickle Cell Disease in adults and children.

9.4 Contraindications for Use

There are no known contraindications for use of the AMICUS Separator except those associated with the infusion of solutions and replacement fluids as required by the apheresis procedure, and those associated with all types of automated apheresis systems.

Use of the AMICUS Separator is contraindicated in those cases where adequate anticoagulation cannot be achieved.

9.5 Materials and Supplies

An AMICUS Operators Manual, Volume 5, RBCx (REC-009200) will be provided. Also, the cleared AMICUS Operator's Manual, Volume 1, Operation Basics (07-19-07-291) will be provided. This volume provides information for the general care and use of the AMICUS Separator System. All information on traceability of materials (i.e., lot numbers) will be documented.

9.5.1 Investigational Materials

The following investigational materials are intended for use in this study:

- FDA cleared AMICUS Separator, Codes 4R4580 or 4R4580R with investigational Software Version 5.0. FTX1708.
    - The AMICUS Separator includes spool (PN# 0112682396) and spool holder (PN# A10418001 C) cleared for use in TPE procedures.
- AMICUS Exchange Kits, Code R4R2339 cleared for use in TPE procedures will be labeled as clinical inventory with FTX1709. The cleared kit is labeled, "For Therapeutic Plasma Exchange (TPE) Procedure," and as such, use of the kit in the evaluation of the RBCx System is a new indication for use.
- AMICUS Waste Transfer Set FTX 1707, Lot #XXXX, Qty XX
- AMICUS Separator Operator's Manual, Volume 5, RBCx (REC-009200)

9.5.2 FDA Cleared Materials

The following FDA cleared materials intended for use in this study may include, but are not limited to:

- Blood Component Filter Set with Vented Spike and Luer Adapter, R4C2170 or equivalent
- Plasma Transfer Set, Code 4C2240 or equivalent
- Apheresis needle with Masterguard Protector, Code 4R2441 or equivalent
- Replacement RBC Units: Additive Solution (AS) RBCs, CPD RBCs, CP2D RBCs, CPDA-1 RBCs, Washed RBCs, other, or comparable as defined by the prescribing physician based on the subject's medical need.
- 0.9% Sodium Chloride for Injection USP 1L, Code 2B1324X or equivalent
- Anticoagulant Citrate Dextrose Solution (ACD) USP Formula A, 1L, Code 4B7891X or comparable; custom anticoagulant may be used and should be defined by the prescribing physician based on the subject's medical need.
- Transfer Pack, any size or code as needed
- Product Sample Packs, Code 4R2335H or equivalent

- Sampling devices or equivalent
- Terumo Sterile Connection Device SCD 312 or equivalent
- Sebra Tube Sealer or equivalent
- Sample Sites, Code 1C8333 or equivalent
- Fresenius Kabi 10 L Waste Bag, Code 9006281 or equivalent
- AMICUS Separator Operator's Manual, Volume 1, Operation Basics, Product Number 07-19-07-291 or equivalent

9.6 Labeling

Appropriate labels for the following materials are included in Appendix B.

- AMICUS Separator, FTX1708
- AMICUS Exchange Kits, FTX1709
- AMICUS Waste Transfer Set, FTX 1707
- Exchange waste red blood cell material containers
- Depletion waste red blood cell material containers

9.7 Method of Assignment to Experimental Article

This study is non-randomized. Each subject will complete one RBC Exchange or RBC Depletion/Exchange procedure using the AMICUS Separator.

9.8 Blinding/Randomization

This study is non-blinded and non-randomized.

10 RISK ANALYSIS

The new RBCx operating protocol is an extension of the indications for use of the AMICUS Separator System. RBCx builds on processes that are already cleared for the AMICUS Separator system including RBC separation and collection, fluid return while maintaining fluid balance, and control and delivery of anticoagulant solution. The system already has proven capability in separating and collecting RBCs (in conjunction with a platelet collection, BK000039) with appropriate control and delivery of anticoagulant solution. The RBCx System is a continuous-flow process that utilizes the same hardware, technological characteristics, and disposable kit as the cleared AMICUS TPE procedure (K111702). Although testing of the investigational

software has been performed in pre-clinical studies and target procedure parameters have been achieved, there is a possibility that the procedure may not achieve the desired results.

10.1 Risks Associated with an Apheresis Procedure

Risks and discomforts that are unique to apheresis procedures may occur. Mild symptoms of hypocalcemia, such as finger or perioral discomfort (e.g., tingling), unusual smell or taste sensation, vibrations, muscle discomfort and/or headache, or paresthesia (abnormal sensation of the skin) may occur temporarily and are caused by citrate in the anticoagulant that is returned to the subject more rapidly than the subject can metabolize it.[4] Study subjects will be monitored for signs and symptoms of hypocalcemia. The return rate may be lowered, the procedure may be paused, or other medical interventions may be indicated by the physician if any of these symptoms occur. Continued rapid administration of citrate anticoagulant may result in more severe evidence of citrate toxicity, including tetany, convulsions or in extremely rare occasions, cardiac arrest and death. Other symptoms, although unlikely, may include skin redness, itching, hives, bronchospasm (difficulty breathing), and abdominal cramps.

On rare occasions, there is a risk of hemolysis, air embolus, blood loss, or blood clotting. In the rare occurrence of hemolysis during a one-time procedure, the subject may experience back pain, nausea, low blood pressure, excess of hemoglobin in the blood plasma, abnormal urine color, and impaired renal function. With chronic therapy, it is possible that chronic hyper-hemolysis in sickle cell subjects may increase the potential for increased blood pressure, higher prevalence of leg ulcers, priapism, and increased pressure in the pulmonary arteries.[7] Air infusion (infusion of >30 mL bolus of air) may interfere with the heart's ability to pump blood from the right ventricle, may disrupt pulmonary blood flow with impaired blood oxygenation, reduce oxygen delivery to the tissues, low blood pressure, chest pain, shortness of breath, lack of oxygen to body tissue, or death (Sponsor's Therapeutics Hazardous Situations and Harms Analysis, 123-RSK-022188). In the rare occurrence of the procedure causing blood clotting during the procedure, the subject may experience severe effects such as stroke, heart attack, kidney problems, or deep vein thrombosis (causing pain, swelling in the affected limb, or redness). In the case of deep vein thrombosis, it is possible for the clot to break off, travel to the lungs, and cause pulmonary embolism. It is important to note that sickle cell subjects are genetically predisposed to the risk of blood clotting. Investigators treating sickle cell subjects should be aware that such patients are at increased risk for blood clots, especially pulmonary embolism.[8] Apheresis technology is designed to monitor for the presence of air, hemolysis, leaks or blockage in the disposable kit during the procedure. The redundant monitoring systems and all the safety features of the AMICUS are the same as in the previously FDA cleared platelet, with concurrent plasma, and/or red blood cell collection, MNC, and TPE procedures.

There are known potential side effects associated with transfusion of blood components that are similar when these components are employed during RBCx procedures. Blood components can result in transmission of infectious agents, even though donated blood is extensively tested.[3]

Subjects will be closely monitored throughout the procedure for any discomfort that may result in more serious reactions such as muscle twitching, convulsions, or in extremely rare occasions, cardiac arrest and death. Subjects will be asked to report any adverse events that they are experiencing during the procedure and approximately 18-24 hours post-procedure.

Although the kit, needles, catheters, and CVAD lines that are used are sterile and proper aseptic technique is employed, in rare cases infection or inflammation may occur. CVADs and lines may also have access complications due to clotting. A transient decrease in cell counts may occur. Decreased coagulation factor concentrations in subjects may occur depending on the RF(s) used and the frequency of therapeutic procedures.

Although testing of the investigational software has been performed during *in vitro* and *in vivo* preclinical studies in healthy subjects and target fluid balance has been achieved, there is a possibility that during the procedure inadequate or excess fluid may be given resulting in hypovolemia or hypervolemia. When blood loss is greater than 15% of the total blood volume, symptoms of hypovolemia may develop resulting in changes to cardiac output, vascular tone, arteriolar contraction leading to reduced blood flow to skin and muscles, and other compensatory metabolic and endocrine responses. When blood loss is greater than 30% of the total blood volume, the majority of subjects will experience circulatory shock due primarily to hypovolemia. Hypovolemia can also occur as a result of too little fluid in the body and may cause the subject to experience rapid heart rate, dizziness upon standing, narrow pulse pressure, apprehension, weakness, nausea, lightheadedness, pallor, thirst, cool skin, and even loss of consciousness.[6] Hypervolemia may occur as a result of too much fluid in the body. Most subjects will pass the excess fluid through their urine; however, subjects with compromised cardiac, pulmonary, and/or renal function may not be able to efficiently complete this process. If they are unable to excrete the excess fluid, it is possible that the fluid may enter the subject's lungs, making it difficult to breathe. These risks may be greater in subjects with SCD since the disease affects the blood's ability to carry oxygen throughout the body; this is especially notable in subjects of lower weight (< 40 kg). To mitigate the risks of fluid imbalance, investigators will ensure subjects are medically stable and should not enroll subjects with compromised cardiovascular or multiple organ diseases who may not tolerate the apheresis procedure. Furthermore, the subject's fluid balance is shown in real-time on the AMICUS procedure display and the operator will monitor the subject's fluid balance during the procedure. The physician will prescribe the appropriate volume of RF as clinically indicated.

Concomitant medication seven days prior to and during the procedure will be documented. Adverse events during the procedure and approximately 18-24 hours post-procedure will be recorded and standard institutional SOPs will be followed to treat subjects who may develop adverse events.

An alternate option for the subject would be not to participate in this study and continue RBCx therapeutic treatments using a commercially available device.

10.2 Risks to Pediatric Subjects

In addition to the apheresis and investigational procedure risks, there may be risks that affect pediatric subjects greater than adult subjects. Maintaining constant fluid balance is important for safe apheresis procedures in pediatric subjects.[9] When the volume of blood loss is fixed, the degree of blood loss is greater in a child than an adult and the effects of fluid and blood loss (listed in Section 10.1 Risk Associated with an Apheresis Procedure) on the circulatory system are greater in a child than an adult.

The Sponsor's Therapeutics Hazardous Situations and Harms Analysis (123-RSK-022188) includes analyses of the AMICUS Separator that evaluated potential harms of fluid imbalance resulting from blood loss and the inappropriate or inadequate infusion of electrolyte or colloid solution replacement fluids (e.g., 0.9% sodium chloride or Ringer's lactate, or colloids, such as albumin solution or fresh frozen plasma). Adjustment of values specific for pediatric patients were made based on weight comparisons between patients (45 kg) and pediatric patients (10 kg). The analysis shows that blood loss at different volumes for pediatric patients will vary from experiences of transient hypovolemia, hypotension, occasional lightheadedness, and occasional temporary loss of consciousness to more serious effects as decreased cardiac output, significant circulatory impairment with possible organ damage, hypovolemic shock, shortness of breath, severe shock, tissue hypoxia, brain damage, cardiac arrest, and death with greater blood loss (>25% loss of blood volume for a pediatric patient). Infusion of excess (>220 mL of electrolyte/colloid solution in addition to the normal replacement volume) or inadequate replacement fluid can lead to hypervolemia and transient hypovolemia, respectively, with the degree depending on the volume and type(s) of fluid infused. However, the significant effect of hypervolemia is unlikely unless cardiovascular status is compromised.

To mitigate the risks of fluid imbalance, investigators will ensure pediatric subjects are medically stable and should not enroll subjects with compromised cardiovascular or multiple organ diseases who may not tolerate the apheresis procedure. Furthermore, the subject's fluid balance is shown in real-time on the AMICUS procedure display and the operator will monitor the subject's fluid balance during the procedure. The physician will prescribe the appropriate volume of RF as clinically indicated.

The pediatric subject's peripheral veins may not be able to accommodate the size of needles necessary for RBCx and therefore may require CVAD lines for adequate flow. Although proper aseptic technique is employed, this may cause infection, thrombosis, or other serious complications. In this study, vascular access will be established as clinically indicated by the physician.

The Sponsor's Hazards and Harms Analysis (123-RSK-022188) includes all risks associated with the RBCx Investigational Device in adult and pediatric subjects.

10.2.1 Risks Associated with Use of the RBCx Investigational Device

The Sponsor has completed a Hazards and Harms Analysis (123-RSK-022188) of all risks associated with the AMICUS RBCx intended use. The analysis includes:[5]

- Potential loss of blood due to leaks
- Thrombosis due to activation of factors by foreign surfaces
- Moderate to severe toxic reaction to citrate anticoagulant (e.g., tetany, seizures, cardiac arrhythmias)
- Damage to RBCs, activation of complement, and denaturation of proteins.
- Potential for sepsis and fever due to bacterial contamination of the subject's returned blood.
- Infectious disease risk to the subject or to the operator due to leaks.
- Electrical shock hazard.
- Subject stress reaction due to removal or loss of blood.
- Air embolism
- Hemolysis

Safety levels for all apheresis-related hazardous situations have been established in alignment with currently accepted industry practice and regulations; these levels have been approved through a cross-functional review including a physician. The Sponsor's documented design and development processes include risk management activities that assess the probability of these hazardous situations, and risk controls are established to maintain device safety. The device is verified to maintain performance within these established safety limits, with results of the verification and risk management activities reviewed through a formal cross-functional design review prior to first human use release. All changes to the device go through the same process of analysis, risk management, verification, and review prior to human use. This process ensures that the original device and all subsequent modifications to the device, do not present unacceptable risks to the subject.

These special controls when combined with general controls (e.g., Good Manufacturing Practice) should mitigate risks associated with the use of the AMICUS Separator with the RBCx software.

The AMICUS instrument hardware including exchange spool and spool holder, disposable AMICUS Exchange Kit, and Blood Component Filter Set with Vented Spike and Luer Adapter to be used in the study were previously cleared by the FDA for AMICUS TPE procedures.

10.3 Demographic Risks

The Sponsor acknowledges the importance of heterogeneity across sex groups in study design and interpretation of primary endpoints. Because beta and alpha globin mutations are located on chromosomes 11 and 16 respectively, and follow the Mendelian laws of genetics for a recessive mutation, the use of the RBC Exchange and RBC Depletion/Exchange in treatment of SCD is not sex associated. Non-sex limited diagnoses prompting RBC Exchange and RBC Depletion/Exchange are found to occur in equal frequency. Additionally, based on the subject evaluation parameters, the AMICUS Separator utilizes programmed algorithms for age and sex-related blood volumes. Therefore, sex enrollment will not be pre-specified. Even though no substantial sex difference is expected, the variability in data across sex groups and its interpretation will be considered. Demographic information, including sex, will be collected and included in the summary statistics.

11 SAFETY EVALUATIONS

11.1 Adverse Events

Each subject will be monitored for signs of adverse events (AEs) and will be asked to provide input as to his/her condition. AEs occurring during the procedure and approximately 18-24 hours post-procedure will be recorded and standard procedures will be followed to treat subjects who may develop adverse events. Up to two attempts to contact the subject by phone will be made approximately 18-24 hours post-procedure. If the site is unable to contact the subject, a certified letter will be sent and the subject will be lost to follow up; however, they will remain evaluable if all other evaluability criteria are met.

AEs will be recorded per Adverse Event Reporting, SOP CLA02011, current issue. All serious adverse events (SAEs) will be recorded per Serious Adverse Event Reporting, SOP CLA02012, current issue.

SAEs and unanticipated device effects (UADEs) related to the device or to study participation will be reported to the EC/IRB and the Sponsor within FDA-specified guidelines.

An AE is defined as any undesirable experience occurring to a subject during a clinical trial whether or not it is considered related to the experimental article.

An adverse device effect (ADE) is an AE related to the use of an investigational medical device.

11.2 Event Severity

The AE will be graded as mild, moderate, or severe. For purposes of consistency, these intensity grades are defined as follows:

ADVERSE EVENT GRADING CRITERIA

Grade	Definition
MILD	Causing no limitation of usual activities. Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
MODERATE	Minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL). Causing some limitation of usual activities.
SEVERE	Medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL. Causing inability to carry out usual activities.

11.3 Episode Pattern

The episode pattern of the AE shall be classified as:

EPISODE PATTERN CRITERIA

Pattern	Definition
SINGLE	The AE occurred one time during use of the investigational device.
INTERMITTENT	The AE occurred two or more times with or without intervention.
CONSTANT	The AE continued consistently during use of the investigational device.

11.4 Action Taken (Procedure)

During the use of the investigational device, an AE may require that some action regarding the procedure be taken. The action taken may be:

1. No change
2. Interrupted
3. Permanently Discontinued
4. Change in procedure parameter(s)

11.5 Action Taken (Subject)

Any treatment or intervention performed for the subject in response to an AE should be documented and an explanation of the intervention provided in the Comment Section.

11.6 Relationship to Device

Any AE will be graded to its relationship to the device. For purposes of consistency these grades are as follows:

ADVERSE EVENT RELATIONSHIP CRITERIA

Relationship	Definition
NOT RELATED	The adverse event can be reasonably attributed to another cause and can be considered unrelated to the study device.
UNLIKELY RELATED	The relationship may or can exist, but other factors may make interpretation difficult. If the relationship cannot be ruled out, it must be considered possible. 1. It may or may not show a reasonable temporal sequence from use of the device. 2. It is likely produced by the subject's clinical condition, environmental or toxic factors, or other modes of therapy administered to the subject, however use of the device cannot be ruled out. 3. It may follow a known or anticipated response pattern to the suspected device.
POSSIBLY RELATED	There is a suggestion of the relationship between use of study device and the adverse event, supported generally, but not conclusively, by the evidence. 1. It follows a reasonable temporal sequence from use of the device. 2. It could not be reasonably explained by the subject's clinical condition, environmental or toxic factors, or other modes of therapy administered to the subject. 3. It follows a known or anticipated response pattern to the suspected device.
RELATED	There is a strong relationship to the use of the study device, which may or may not stop upon discontinuation of the study device use and may reoccur if the subject is re-exposed to the device.

11.7 Relationship to Procedure

Any AE will be graded to its relationship to the procedure. For purposes of consistency these grades are as follows:

ADVERSE EVENT RELATIONSHIP CRITERIA

Relationship	Definition
NOT RELATED	The adverse event can be reasonably attributed to another cause and can be considered unrelated to the procedure.
UNLIKELY RELATED	The relationship may or can exist, but other factors may make interpretation difficult. If the relationship cannot be ruled out, it must be considered possible. 1. It may or may not show a reasonable temporal sequence from the procedure. 2. It is likely produced by the subject's clinical condition, environmental or toxic factors, or other modes of therapy administered to the subject, however the procedure cannot be ruled out. 3. It may follow a known or anticipated response pattern to the suspected procedure.
POSSIBLY RELATED	There is a suggestion of the relationship between use of study procedure and the adverse event, supported generally, but not conclusively, by the evidence. 1. It follows a reasonable temporal sequence from use of the procedure. 2. It could not be reasonably explained by the subject's clinical condition, environmental or toxic factors, or other modes of therapy administered to the subject. 3. It follows a known or anticipated response pattern to the suspected procedure.
RELATED	There is a strong relationship to the use of the procedure which may or may not stop upon discontinuation of the procedure and may reoccur if the subject is re-exposed to the procedure.

11.8 Outcome

The outcome of the AE shall be documented using the following key:

OUTCOME CRITERIA

Outcome	Definition
RECOVERED	The subject fully recovered from the AE without after-effect(s).
RECOVERED WITH SEQUELA(E)	The subject recovered from the AE with after-effect(s) subsequent to the AE.
CONTINUING	The adverse event continued after the procedure was completed.
DEATH	Death due to this AE or other causes.

11.9 Serious Adverse Events

A *serious adverse event* (SAE) is any adverse event that results in any of the following outcomes:

- Death
- Life-threatening
- Disability or permanent damage
- Hospitalization (initial or prolonged)
- Congenital anomaly/birth defect
- Other serious (important medical event)
- Required intervention to prevent permanent impairment or damage (Devices)

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed.

Unanticipated adverse device effect (UADEs) is any serious ADE on health, safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the study protocol, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

All SAEs and UADEs must be reported to the Applied Product Testing Site Management (APT SM) within 24 hours after onset or identification of an event.

12 DATA QUALITY ASSURANCE

12.1 Data Reporting

Sites must fax Screening and Enrollment Logs and redacted copies of source documents labeled with the subject's Subject ID as soon as they become available and at least weekly to (847) 550-2956 to allow the Sponsor to assess study enrollment and procedure evaluability.

12.2 Data Capture System

An electronic data capture (EDC) system will be used for data collection.

12.3 Data Recording

All data provided to the study Sponsor will be encoded to maintain study subject confidentiality. Subject related procedure and AE data will be entered onto worksheets that will serve as source data.

All original laboratory data (such as laboratory test results, instrument print-outs, laboratory results, notebook entries, etc.) must be retained by the investigator or designee for a minimum of two years or as deemed by the Sponsor and be accessible by cross-reference to laboratory notebooks, hospital medical records, subject history summary, etc.

12.4 Electronic Case Report Forms (eCRF)

All entry of study data into the Electronic Data Capture (EDC) system will be performed by authorized and trained personnel from the study site, who will receive a secure login and password from the Sponsor. Data will be entered from source documentation into the EDC system. The EDC system will generate automatic queries defined in the Data Checks Specification document, as data are entered and saved in the EDC database. Applied Product Testing Data Management (APT DM) may perform additional manual review of the data to ensure that data are within the pre-defined criteria and may generate manual queries as necessary. Applied Product Testing Statistician (APT Stats) and APT SM may also identify additional manual queries during the review of the data. Authorized personnel from the site will need to address each query in a timely matter.

After data are entered by the study site personnel, source data verification (SDV) will be performed by the Sponsor or Sponsor designee. APT SM will review responses to automated

electronic data checks. Data that are changed after verification will be re-verified by APT SM until all pages are completely verified.

At the completion of the study, the electronic data will be archived using a digital video disc (DVD).

12.5 Monitoring Procedures

The Sponsor will perform periodic centralized and remote monitoring, at least once prior to the interim report and at least once prior to the final report, in addition to the initiation and close out visits. Additional site/monitoring visits may be performed as necessary. The monitor's on-site visit will be recorded by the monitor on the Site Visit Log kept at each study site. A site monitoring plan is available from the Sponsor upon the site's request.

The monitor will compare data entered into the eCRFs with source documents (i.e., laboratory, hospital, or clinical records). At a minimum, source documents must be available to substantiate subject identification, eligibility, proper informed consent procedure, visit dates, adherence to the protocol, adequate reporting, and follow up of all adverse events. Specific items required as source documents will be reviewed with the study staff prior to the study. Discrepancies following the review of the eCRFs and source documents will be discussed with investigational personnel.

The monitor will review study documents including, but not limited to, EC/IRB approvals and correspondence, ICF for completeness, compliance with 21 CFR 52, and to ensure the correct ICF version is being used. The monitor will also ensure PI compliance and accountability of investigational inventory.

The Sponsor expects that relevant study personnel, source documents and a suitable environment for review of study-related documents will be available to the monitor at the time of monitoring visits. The monitor will communicate with the investigator periodically during the study to provide feedback on the study conduct.

12.6 Maintenance of Records

Documents that must be provided to the Sponsor prior to any site initiating the study include but may not be limited to:

- Medical Device Agreement
- Financial Disclosure and Arrangements of Clinical Investigator
- Investigators Signed Protocol Signature Sheet
- Study Site Personnel Curriculum Vitae (CVs) and Medical Licenses

- A copy of the EC/IRB-approved ICF and other adjunctive material (e.g., marketing flyers) to be used in the study, including written documentation of EC/IRB approval for these materials.
- Name and address of the EC/IRB, a statement that it is organized and operates according to GCP and applicable laws and regulations or the Federal Wide Assurance (FWA) number and list of the EC/IRB's current members.

12.7 Record Retention

During or after the study, the appropriate regulatory agency may inspect study records at the investigational site. Since inspection may arise at any time, records should be kept current at all times. The Sponsor must be advised of any request by a regulatory agency to visit or inspect the site. Records must be maintained for:

a. A period of at least two years after the last approval of a marketing application in an ICH region when there are no pending or contemplated marketing applications in an ICH region.
b. A period of at least two years after the formal discontinuation of development of the experimental article.

These documents should be retained for a longer period of time if required by the applicable regulatory requirement(s) or if required by the Sponsor.

13 STATISTICAL METHODS AND SAMPLE SIZE

13.1 General Overview

The RBC Exchange and RBC Depletion/Exchange procedures have the same therapeutic endpoint, FCR. The RBC Depletion/Exchange procedure is a modification of the RBC Exchange procedure that automates the transition between the depletion phase and the exchange phase of the procedure.

This is a multicenter, single-arm, open label study. Hypothesis testing will be performed to evaluate the primary objective of the 95% confidence interval around the mean ratio of actual to target FCR as measured by Hemoglobin S in the subjects pre- and post-procedure to a pre-defined FCR range of 0.75 to 1.25.

Hypothesis testing is not planned for secondary variables. Summary statistics (mean, standard deviation, median, minimum, maximum and count) will be presented for continuous parameters with their 95% confidence limits as applicable. Discrete parameters will be presented by total number and percentage. A detailed statistical procedure will be outlined in the Statistical Analysis Plan.

Any change to the data analysis methods described in this protocol will require an amendment only if it changes a principal feature of the protocol. Any other change to the data analysis methods described in the protocol, and the justification for making the change, will be described in the final study report.

The overall study success for testing the hypothesis of equivalence is that the 95% confidence interval around the AMICUS mean ratio of the FCR actual to target is within the pre-defined range of 0.75 and 1.25.

13.2 Determination of Sample Size

Sample size determination is based on historical *in vitro* data from evaluable RBC Exchange and evaluable RBC Depletion/Exchange AMICUS procedures for the therapeutic endpoint of the mean ratio of FCR. The margin of 25% was chosen to allow for the known intrinsic variability of the RBCx procedure, principally in actual Hct of replacement RBCs, total blood volume of patients, Hb S assay and intraprocedure mobilization of RBCs.[9] The sample size needed for the study design is given as:[10]

$$N= 2(Z_1-\alpha + Z_1-\beta)^2\ \sigma^2\ \div \delta^2$$

Where $Z_1-\alpha$ and $Z_1-\beta$ are the standard normal deviates corresponding to 1 minus significance level (α) and 1 minus the type II error (β) respectively, σ^2 is the variance or squared standard deviation of the outcome and δ is the margin.

Since we planned to have a 90% power at the 0.025 significance level to detect equivalence to a ratio of 1, then the sample size with the margin of 0.25 and observed standard deviation of the difference of 0.43 with β =0.10 will yield:[10]

$$N= 2(1.96 +1.28)^2\ 0.43^2 \div .25^2 = 2(10.4976)\ 0.185 \div 0.0625 = 62.$$

Therefore, a sample size of 62 evaluable procedures will be used to demonstrate that AMICUS is within a margin of 25% with at least 95% (two sided) confidence and at least 90% power.

13.2.1 Analysis Data Sets

Formal hypothesis testing per the sample size statement will be performed for the primary endpoint across investigational sites including the all evaluable population. This population is defined as those subjects who successfully complete the AMICUS Separator RBCx procedures and have all values for the primary endpoint calculation reported by the site. The primary endpoint is calculated based on actual FCR as measured by Hb S in subjects pre- and post-procedure as well as the target FCR. Subjects will be analyzed as treated and missing values will not be imputed.

The primary analysis of the all evaluable population will establish that the analysis for the accuracy of the AMICUS Separator as measured by 95% confidence interval around the mean ratio of the FCR actual to target range is within the pre-defined range of 0.75 and 1.25.

In addition, a second analysis of an Intent-to Treat (ITT) will be performed that will consist of all enrolled subjects. Every effort will be made to obtain missing data. The pre- and post-procedure Hb S values used to calculate the primary endpoint, Fraction of Cells Remaining, are laboratory measurements. Several potential reasons for missing laboratory data include lost, broken, and mislabeled samples or technical issues. Missing values in the primary endpoint are assumed to be a random event, since the fact that they are missing is unrelated to the measured parameter, Hb S. The ITT analysis, will be analyzed imputing the missing value with the observed mean.

A sensitivity analysis will also be conducted to evaluate the impact of the imputed missing data.

Secondary objectives will be evaluated using RBC Exchange and RBC Depletion/Exchange summary statistics (mean, standard deviation, median, minimum, maximum, and count).

13.2.2 Safety Analysis

Safety measures that will be summarized in this study include subject and device related SAEs. Subject AEs will be recorded during each procedure and approximately 18-24 hours post-procedure. AEs will be presented using the Description of Event key on the Case Report Form (CRF). Events classified as *Other* will be presented as verbatim terms. AEs occurring multiple times per subject will be counted once for subject-reported frequencies, where the highest severity or least favorable relationship will be assumed. The following adverse event analyses will be performed:

- The frequency of subjects who reported AEs and the frequency of AEs reported by and across severity, seriousness, and relationship to study test article will be presented across and by investigational sites overall
- The frequency of subjects who reported AEs and the frequency of AEs reported will be presented for each adverse event type across and by investigational sites overall.

AEs will be presented for All Subjects Enrolled and Evaluable Subject populations. The action taken with respect to the procedure, any action taken to treat the subject, and outcome will be recorded. The adequacy of the performance of the AMICUS system during the RBCx procedure will be assessed through documentation of alarms or UADEs.

13.3 Study Endpoints and Methodology

The study endpoints include the primary and secondary objectives. This is a multicenter, single-arm, open label study. Hypothesis testing will be performed to evaluate the primary objective of

the 95% confidence interval around the mean ratio of actual to target FCR as measured by Hb S in the subjects pre- and post-procedure to a pre-defined FCR.

Hypothesis testing is not planned for secondary variables.

The calculation for the primary endpoint ratio is given by the formula for the AMICUS Separator RBC Exchange and RBC Depletion/Exchange procedures in the efficacy evaluable subject population as

$$Target\ FCR = \frac{Desired\ End\ HbS\ Value\ (\%)}{Measured\ PreProcedure\ HbS\ Value\ (\%)}\ x\ 100\%$$

$$Actual\ FCR = \frac{Measured\ PostProcedure\ HbS\ Value\ (\%)}{Measured\ PreProcedure\ HbS\ Value\ (\%)}\ x\ 100\%$$

The resultant ratio would be FCRa ÷ FCRt.

A two-sided hypothesis test is given as:

$$H_0:\ P_T\,LL<0.75\ \text{ and/or }\ P_T\,UL>1.25$$

Versus

$$H_1:\ 0.75 \leq LL\ P_T\ UL \leq 1.25$$

Where P_T = the 95% CI for AMICUS mean ratio and LL = Lower limit and UL = Upper limit

We will reject H_o at the alpha level of significance if the entire 95% confidence interval around the mean ratio is within the predefined range. Stated explicitly, if the lower limit of the 95% confidence interval around the mean ratio is greater than or equal to the lower predefined range boundary (0.75) and simultaneously the upper limit of the 95% confidence interval around the mean ratio is less than or equal to the upper predefined range boundary (1.25).

Summary statistics (mean, standard deviation, minimum, median, maximum, and count) of the RBC Exchange and RBC Depletion/Exchange procedure data will be used to evaluate the secondary objectives: evaluation of the accuracy of subject End Hematocrit, accuracy of Fluid Balance, subject cellular loss post-procedure, and serious device related adverse events.

For the primary and secondary analyses, there are circumstances during an RBC Exchange or RBC Depletion/Exchange procedure that may cause one of the parameters to be non-evaluable. Procedures with these irregularities will not be evaluable for the affected procedure parameter.

The overall study success for testing the hypothesis of equivalence is that the 95% confidence interval around the AMICUS mean ratio of the FCR actual to target is within the pre-defined range of 0.75 and 1.25. Data from all sites will be pooled for the hypothesis test. Descriptive statistics will be presented for the primary endpoint ratio by site.

A detailed statistical procedure will be outlined in the Statistical Analysis Plan. All data analyses will be performed using SAS, Cary, NC version 9.4.

13.4 Criteria for End of Study or Stopping Rules

Upon completion of approximately 62 evaluable subjects the sites will be notified to stop enrollment.

13.5 Evaluability Criteria

- If the procedure is terminated prior to completion, the procedure is non-evaluable.
- If reinfusion is performed, the post-procedure blood sample must be drawn prior to reinfusion during any study procedure.
- The operator should not change the following parameters after the procedure start, Subject Hct, Avg RF Hct, or Total Blood Volume.
- If a saline bolus is given during the procedure, END Hct is not evaluable.
- The procedure is dependent on accurate input data.
    - If the mean of the measured hematocrit of the RF units transfused and the entered Avg RF Hct used differs by more than five (5) hematocrit points, then data for FCR and END Hct are non-evaluable.
    - If the subject hematocrit input value and the actual day of treatment pre-procedure hematocrit differs by more than five (5) hematocrit points, then data for FCR and END Hct are non-evaluable.
    - If the subject day of treatment pre-procedure Hb S value results in a calculated Target FCR that differs by more than six (6) FCR points from the entered Target FCR, then data for FCR is non-evaluable.

All procedures performed during this study will be assessed for evaluability using the current criteria. Parameter non-evaluability will not result in the procedure being non-evaluable for other analyses. A detailed statistical procedure will be outlined in the Statistical Analysis Plan. All data analyses will be performed using SAS 9.4 Procedure Guide, Second Edition, Volumes 1-4 (2013).

The primary endpoint will be assessed for the evaluable subject population in this study. This population is defined as those subjects who successfully complete the AMICUS Separator procedures and values for all parameters of the RBC Exchange formula are reported (e.g. day of procedure pre- and post-procedure Hb S results are obtained) and all evaluability criteria are met.

All other parameters will be analyzed with summary statistics (mean, standard deviation, median, minimum, maximum and count) presented for continuous parameters with their 95% confidence limits as applicable. Discrete parameters will be presented by total number and percentage.

13.6 Subject Disposition

Subject disposition and reasons for study or study procedure discontinuation will be listed and summarized in the final report.

13.7 Subject Demographics and Baseline Characteristics

Subject demographics and baseline characteristics will be collected and summarized in the final report.

13.8 Interim Analysis

An interim analysis is planned when approximately half the subjects are enrolled and will include summary statistics only, without Hypothesis Testing of the primary objective. The interim report will summarize the details of the subjects enrolled, laboratory data, safety data, device related serious adverse events and results of the evaluable procedures.

The investigational study will initially enroll up to 60 subjects who are 18 years of age and older to obtain approximately 31 evaluable procedures. After approximately 31 evaluable subjects have completed the study, pre- and post-procedure safety data including vital signs (blood pressure, temperature, pulse, and respiration rate), hematology results, AEs including SAEs, and UADEs will be summarized for all subjects enrolled. An independent DSMB composed of transfusion medical experts, including physicians familiar with RBCx treatment of patients will be established by the Sponsor for this study. The DSMB will review summary statistics and reported subject safety data to determine if it is safe to begin enrollment in a pediatric population. Following review by the DSMB, the interim report will be submitted to the FDA for review as an IDE supplement. The supplement must be approved by the FDA prior to enrolling pediatric subjects six to 17 years old. In addition to FDA approval, the results of the DSMB will be sent to the EC/IRBs to obtain approval to start with pediatric subjects.

The interim report will be submitted to TÜV for the purpose of CE marking.

14 ADMINISTRATIVE RULES

14.1 Investigator's Responsibilities

The study will be conducted in a manner designed to assure the acceptability of the data by regulatory authorities. All investigators and sub-investigators, as defined in 21 CFR 54.2(d), are required to complete a Disclosure of Financial Interest supplied by the Sponsor. This section contains a summary of investigator responsibilities that will help ensure the study is performed in compliance with regulations. Specific mechanisms and procedures are described to maintain the compliance of both the investigator and the Sponsor.

Certain events in the performance of the study require particular reports from the investigator within prescribed time frames. Refer to Table 7 for a summary of the required investigators' reports.

Table 7. REQUIRED REPORTS FROM INVESTIGATOR

Report	Submit To	Time
Withdrawal of EC/IRB approval	Sponsor	Within 5 working days
Progress Report(s)	Sponsor EC/IRB	Regular intervals (at least yearly)
Deviation from protocol	Sponsor EC/IRB	As soon as it becomes apparent but no later than 5 working days
Failure to obtain informed consent	Sponsor EC/IRB	As soon as it becomes apparent but no later than 5 working days
Final Report (Completed Case Report Forms constitute a Final Report from Investigator)	Sponsor EC/IRB	Within 3 months after termination
Both Serious and Unexpected Adverse Events 21CFR 312.64	Sponsor	As soon as it becomes apparent but no later than 24 hours by telephone, facsimile or email
	EC/IRB	As soon as it becomes apparent but no later than 10 working days
Both Serious and Unexpected Adverse Device Events 21CFR 812.150	Sponsor	As soon as it becomes apparent but no more than 24 hours by telephone, facsimile or email
	EC/IRB	As soon as it becomes apparent but no later than 10 working days

14.2 Confidentiality

The investigator and his/her staff will agree to treat all aspects of the study in a confidential manner. All documents and data pertaining to the study can only be released with the consent of the Sponsor or as required by federal or state law or authorized regulatory agencies. Each investigator agrees to permit review of all pertinent data by the Sponsor or its agents as well as representatives of the FDA, or other appropriate regulatory agencies during and/or following performance of the study.

14.3 Protocol Modifications

All protocol amendments must be issued by the Sponsor, signed and dated by the investigator and should not be implemented prior to EC/IRB approval except where necessary to eliminate

hazards to subjects or when the change(s) involve only logistical or administrative details (e.g., address or telephone changes).

14.4 Protocol Deviations

In situations regarding a departure from the protocol, the investigator will contact the appropriate Sponsor representative by fax or telephone. Notification to the Sponsor should be made prior to implementing a departure from the protocol, if at all feasible. In all cases, contact with the Sponsor should be made as soon as possible in order to discuss the situation and agree on an appropriate course of action. The protocol deviation log will describe any departure from the protocol and the circumstances that necessitated the departure. Protocol deviations will be entered into the database.

14.5 On-Site Audits

Representatives of the Sponsor may visit the investigational site to carry out an audit of the study in compliance with regulatory guidelines and company policy. Such audits will require access to all study records, including source documents, for inspection and comparison with eCRFs. In accordance with HIPAA, as applicable, subject privacy will be respected at all times. Sufficient prior notice will be provided by the Sponsor to allow the investigator time to prepare for the audit.

The governing EC/IRB may conduct an on-site audit during the course of the study. The investigator should notify the Sponsor, if they have been contacted by the EC/IRB regarding an audit.

Similar auditing procedures may be conducted by agents of any regulatory body reviewing the results or procedures of this study. The investigator should immediately notify the Sponsor if they are contacted by a regulatory agency concerning an inspection.

14.6 Ethics

14.6.1 Institutional Review Board

The investigator will be responsible for obtaining Ethics Committee (EC)/Institutional Review Board (IRB) approval for the study as appropriate and for either completing the EC/IRB Certification form (provided to the investigator) or providing the Federal Wide Assurance (FWA) number. After approval of the protocol, ICF, and any other adjunctive material (e.g. marketing flyers) by the EC/IRB, the investigator must provide a copy of the EC/IRB approval document and the completed EC/IRB Certification form or assurance number to the Sponsor. Copies of these documents are to be maintained at the study site. The completed EC/IRB

Certification form verifies that the review board complies with federal regulations as described in 21 CFR Part 56.

The written notification must be signed by the Chairman of the EC/IRB, or his/her designee and must identify the specific protocol. In cases where an EC/IRB member has a specific conflict of interest, abstention of the individual from the vote should be documented. An investigator or sub-investigator may be a member of the EC/IRB, but may not participate in the deliberation or vote on any research in which he/she is involved.

14.7 Informed Consent

The ICF, or assent when applicable, will be in accordance with the Declaration of Helsinki, principles of GCP, applicable regulatory requirements, subject privacy requirements and the Sponsor's policies. The ICF, and assent when applicable, will include the subject's age and the time consent was signed by the subject. The subject will provide a current working phone number on the ICF so that the site can assess adverse events approximately 18-24 hours post-procedure. The ICF that is used must be approved by both the Sponsor and the reviewing EC/IRB. Each subject, or legally authorized representative, must provide written informed consent, or assent when applicable, according to local requirements after the nature of the study has been fully explained in order to participate in the study. The ICF must be signed prior to performance of any study-related activity.

The investigator, or designee, must explain to potential subjects, or their legal representatives, the aims, methods, reasonably anticipated benefits, potential hazards and risks of the study, right to refuse participation and right to withdraw without affecting the subject's medical care. The other elements of the informed consent will be explained and subjects will be given the opportunity to ask questions. After this explanation, but before entry into the study, consent should be appropriately documented by the subject, or his/her legally authorized representative's dated signature. If a subject, or his/her legal representative, is unable to read or requires a translator, an impartial witness must be present during the entire informed consent discussion and process. The signature of the impartial witness will certify the subject's consent. The subject, or his/her legal representative, will be given a signed and dated copy of the ICF.

14.8 Experimental Article(s)

Any experimental article under evaluation must be kept under strict control and used only as described in the protocol. The investigator will be asked to ensure that the experimental article(s) is stored and returned/destroyed appropriately and to provide documentation in support of this.

All experimental article(s) will be labeled according to regulatory requirements. Experimental article(s) must be stored under conditions set forth by the Sponsor in this protocol, on the product label, or communicated in writing. The experimental article must be stored in a secure area segregated from non-experimental articles.

If an experimental article(s) fails to perform in the expected manner, the investigator will notify the Sponsor immediately.

14.9 Documentation of Laboratory Methods and Test Results

Prior to the start of a study, the Clinical Research Associate (CRA) may visit the investigator's laboratory to evaluate the facility, personnel and the subject population available for study.

The investigator is required to have SOPs in place for routine tests performed in the investigator's laboratory. Quality control measures should be in place for routine tests performed in the investigator's research laboratory to ensure the acceptability of test results. If a routine test method must be modified during the study, the nature and reasons for the modifications and a statement of scientific soundness must be provided by the investigator.

When ancillary laboratory facilities are used by the investigator (e.g., hospital clinical laboratory) to perform particular tests, that laboratory should be licensed or accredited for the tests being performed in the study under the current Clinical Laboratories Improvement Act (CLIA), the College of American Pathologists (CAP), state licensure, or other acceptable certifying body.

If a new test procedure is being used in the study, literature and documentation of the test method development and a validation of suitable scientific rigor performed in the investigator's laboratory must be documented. The new test method development should include a suitable quality control procedure to ensure the acceptability of test results from each run. If the new test method must be modified during the study, the nature and reasons for the modifications must be documented by the investigator.

14.10 Publications

The protocol, procedures, and data pertaining to this study will be treated as confidential information. Publication of data and/or information derived from these studies must be done with the prior review and approval of the Sponsor. Sponsor personnel may share authorship with investigators on abstracts, oral presentations, and manuscripts. The principal author will be the person assuming primary responsibility for the abstract or manual.

15 REFERENCES

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7. Taylor JG, Nolan VG, Mendelsohn L, Kato GJ, Gladwin MT, Steinberg MH. Chronic Hyper-Hemolysis in Sickle Cell Anemia: Association of Vascular Complications and Mortality with Less Frequent Vasoocclusive Pain. PLoS ONE 3(5): e2095, 2008.

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Appendix A. INVESTIGATOR APPROVAL

Investigator's Approval

I have reviewed the Fresenius Kabi USA LLC Protocol AMIC-003-CMD Evaluation of the AMICUS Red Cell Exchange System in Sickle Cell Patients. I have fully discussed the objective of this study and the contents of this protocol with the Sponsor's representatives.

I confirm that I have read and understand this protocol. I agree to conduct this study in accordance to this protocol, the applicable FDA Code of Federal Regulations (21 CFR Parts 50, 54, 56, and 812), ISO 14155:2011 (E) and World Medical Association Declaration of Helsinki (2013 Version), the ICH Tripartite Guideline for Good Clinical Practice (GCP), the privacy regulation of the Health Insurance Portability and Accountability Act of 1996 (21 CFR Parts 160 and 164), any local regulations and with the terms outlined in the Study Agreement.

I will accept the Sponsor's oversight of the study. I have read and understand all related information and guides provided by Fresenius Kabi USA LLC, as applicable. I will abide by the publication plan set forth in my agreement with Fresenius Kabi USA LLC, and will promptly submit the protocol to the EC/IRB.

_____ _____ _____

Principal Investigator (Print Name) Signature Date

_____

Institution

Appendix B. LABELS

Labeling

The AMICUS Separator will display a label with the following text:

Caution: Investigational device limited by United States law to investigational use.

FTX 1708 AMICUS Separator
This AMICUS Separator contains Investigational Software Version 5.0.

The AMICUS Exchange Kits will contain a label with the following text for US sites only:

Caution: Investigational device limited by United States law to investigational use.

FTX 1709 AMICUS Exchange Kits

The AMICUS Waste Transfer Set will contain a label with the following text for US sites only:

Caution: Investigational device limited by United States law to investigational use.

FTX 1707 AMICUS Waste Transfer Set
Lot#: XXXX, Exp: XXXX, Qty: XX

Store at Controlled Room Temperature.
Protect from Freezing. Avoid Excessive Heat.

An additional label will be applied for the AMICUS Exchange Kits, AMICUS Waste Transfer Sets, and AMICUS Separator for Non-US Sites:

Caution: Investigational device limited by United States law to investigational use.

AMIC-003-CMD: Evaluation of the AMICUS Red Blood Cell Exchange (RBCx) System in Sickle Cell Patients

The waste container(s) will be labeled with the following text for all sites:

For Laboratory Use Only – Human blood collected into these containers or blood components prepared from this blood must not be transfused into humans. Not for transfusion.

EXCHANGE WASTE RED BLOOD CELL MATERIAL

Subject Study ID#:_____Date: _____

For Laboratory Use Only – Human blood collected into these containers or blood components prepared from this blood must not be transfused into humans. Not for transfusion.

DEPLETION WASTE RED BLOOD CELL MATERIAL

Subject Study ID#:_____Date: _____

Appendix C. SAMPLE INFORMED CONSENT AND ASSENT

SAMPLE INFORMED CONSENT FORM

Italicized writing is for guidance only and should not be included in the final document.

Adult Consent to Participate in a Research Study

Investigators at *(institution's name)* invite you to consider taking part in a research study called:

AMIC-003-CMD
Evaluation of the AMICUS Red Blood Cell Exchange (RBCx) System in Sickle Cell Patients

Sponsored by Fresenius Kabi USA, LLC and carried out by *(investigator's name).*

You are being asked to be in a research study of a medical device known as the AMICUS Separator.

The purpose of this form is to help you decide if you want to be in this study. Please read this form carefully. It may contain words that you do not understand. Please ask the study doctor or nurse to explain any words or information that you do not clearly understand. You may take home an unsigned copy of this form to think about or discuss with family or friends before making your decision.

If you sign this form, you are giving your permission (or consent) to be a part of this study. You can agree to take part in this study and change your mind later without any negative effects.

You should not sign this form if you have any questions that have not been answered.

I. INTRODUCTION

Blood is made up of four parts: plasma, platelets, white blood cells, and red blood cells. Hemoglobin is a protein found in the red blood cells. It carries oxygen from the lungs to other parts of the body.

You are being asked to take part in this study because you have a hemoglobin disorder called sickle cell disease. Hemoglobin disorders are passed down through families. They are known for abnormal production and shape of hemoglobin proteins. Abnormal hemoglobin production and shape interfere with the body's ability to circulate blood and

carry oxygen throughout the body. This causes a variety of problems and symptoms that you may have.

Removing red blood cells (which contain abnormal hemoglobin) and replacing them with healthy red blood cells or another fluid is a procedure called red blood cell exchange (also called RBCx). RBCx is used to treat people with some hemoglobin disorders.

II. WHY IS THIS STUDY BEING DONE?

This study is being done because a new procedure, red blood cell exchange (also called RBCx) has been added to the AMICUS Separator.

The AMICUS Separator is a blood collection machine called an apheresis device. It collects blood from the patient. As the blood is collected, the device separates it into its four different parts: plasma, platelets, white blood cells, and red blood cells. The device removes the unwanted blood parts and returns some of the other parts back to the patient along with healthy donor blood and/or another fluid, like salt water solution (also called saline), to replace what was removed. This is called the replacement fluid. Replacement fluid is needed to keep the patient's blood level normal.

In RBCx, the red blood cells that are removed from you are replaced with a new fluid that your doctor will prescribe. The doctor may use healthy red blood cells or a mixture of other fluids that do not contain blood (like saline) and blood to replace the red blood cells that were removed.

The parts of the blood that are removed (also called waste blood material) and any samples taken are tested and then discarded after the testing is finished.

The AMICUS Separator can perform similar types of exchange procedures based on the type of replacement fluid the doctor prescribes. This study will look at two types of RBCx procedures: RBC Exchange and RBC Depletion/Exchange.

The doctor will prescribe the type of RBCx procedure you need based on your medical condition.

The AMICUS Separator is approved for other treatment procedures. It is also approved for several blood collection procedures in healthy donors. The AMICUS Separator is **not** approved to perform RBCx procedures. Any RBCx procedures performed on the AMICUS Separator are for research purposes only.

III. SUBJECT SELECTION

You can take part in this study if:

- You are 6 years old or older.
- You have sickle cell disease and require RBC Exchange or RBC Depletion/Exchange treatment.
- You are medically stable and have been previously treated with an RBC Exchange or Depletion/Exchange procedure.
- You give informed consent by signing this form before you begin any activities related to this study. If you cannot give your informed consent, your legal representative can give it for you.
- Your doctor has enough RBC replacement products that are right for your blood type.
- The doctor or nurse can access your veins well enough to run the RBCx procedure.
- You are able to tell the doctor or nurse how you are feeling.
- You agree to tell the operator, doctor, and/or nurse any bad side effects you feel during the procedure and by telephone about 18-24 hours after the procedure.

You cannot take part in this study if:

- The RBCx treatment is done during a hospital emergency visit.
- You were released from the hospital less than a week ago.
- You are not able to fully understand the rules of the study and give informed consent and do not have a legal representative who can provide it for you.
- Your doctor feels there are factors (such as drug or alcohol abuse, etc) that could interfere with your ability to follow his or her instruction or the rules of the study.
- You have experienced a bad side effect with an RBCx procedure in the past.
- If your doctor feels you have a life expectancy fewer than 30 days.
- You refuse blood products.
- You are pregnant.
- You do not follow the doctor’s instructions to stop any medicine that he or she tells you to while taking part in this study.

IV. WHAT IS INVOLVED IN THE STUDY AND HOW LONG WILL I BE IN THE STUDY?

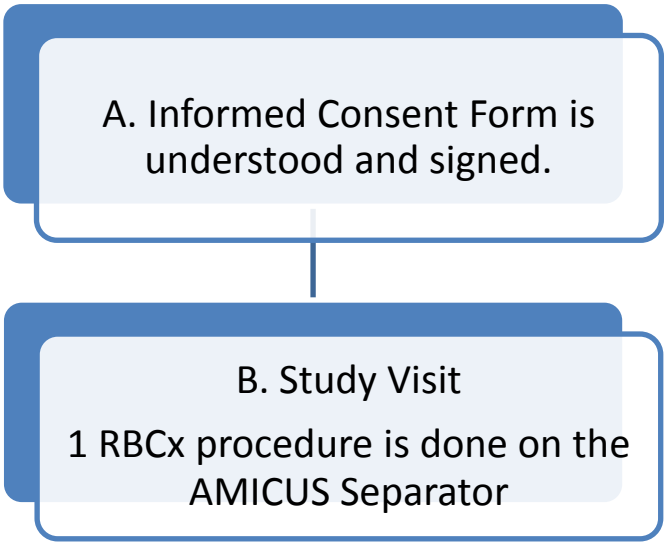
You will be 1 of up to 130 subjects taking part in this study at up to 7 study sites.

Your doctor will prescribe the type of RBCx procedure that will be used:

- RBC Exchange
- RBC Depletion/Exchange

You will be required to complete 1 RBCx procedure at the Study Visit.

The following figure shows how this study is designed:



RBCx Study Design

During the Study Visit, your doctor or nurse will collect information from you. Your date of birth, age at the time of signing this form, sex, ethnicity, race, and sickle cell diagnosis will be documented. Your height and weight will be collected.

Medicines you have taken up to 7 days before the Study Visit and during the procedure will be recorded. These will include medicines that have been prescribed to you by a doctor and medicines that do not need a prescription.

Before the start of the procedure, your doctor or nurse will measure your blood pressure, temperature, pulse, and breathing rate. About 20 milliliters of blood (about 1 ½ tablespoons) will be taken from you to measure your blood cell counts, test your hemoglobin, hematocrit, and plasma hemoglobin (to determine if red cells have broken). If you are female with child bearing ability, you will also be required to take a pregnancy test at the beginning of the Study Visit. If you find out you are pregnant during the study and up to 30 days after the procedure is completed, you have to tell the study doctor.

Two (2) of your veins will be accessed using the method your doctor or nurse prescribes. In an RBCx procedure, your blood will remain within a sterile, single use kit during the procedure. Blood will be taken from one vein and the device will separate it into its 4 parts. Your red blood cells will be collected in a plastic bag (waste blood material). Healthy donor red blood cells will be returned to you along with some of your plasma and blood cells in the other vein. Depending on your sex, height, weight, and doctor's orders, about 3-5 liters of your blood will be processed and returned to you during the RBCx procedure. Only a portion of your blood will be removed at one time during the RBCx procedure.

After the procedure is finished, the doctor or nurse will measure your blood pressure, temperature, pulse, and breathing rate again. They will also take about 20 milliliters of blood (about 1 ½ tablespoons) to measure your blood cell counts, test your hemoglobin, hematocrit, and plasma hemoglobin.

The waste blood material will be tested after the procedure is finished. All samples and waste blood cells removed will be discarded after testing is finished.

For the RBC Depletion/Exchange procedure, about half way through the procedure, the doctor or nurse will measure your blood pressure, temperature, pulse, and breathing rate again. They will also take about 15 milliliters of blood (about 1 tablespoon) to measure your blood cell counts, test your hemoglobin, hematocrit, and plasma hemoglobin.

If your doctor or nurse cannot start the procedure for certain reasons, you may come back one more time to try again. This may happen if your doctor or nurse cannot access your veins or if there isn't enough blood that is right for you. If you have an emergency RBCx procedure after you have agreed to participate in the study, it will not be counted as part of the study. You will be able to come back another time to complete the study procedure.

Your current working telephone number is:

_____(**enter current working phone number and area code**). A member of the study team will call you about 18-24 hours after the procedure is done to see how you are feeling. The study team will try to call you up to 2 times. They will ask you how you are feeling. You will tell them how you are feeling. If the study team is not able to reach you, they will send you a letter in the mail.

An electronic database will be used. Your name will not be entered into the database. You will be given a unique subject identification number that will be used in place of your name. Your date of birth will be entered into the electronic database. The database will be password protected and only approved people will have access to the database.

V. ARE THERE BENEFITS (GOOD THINGS) TO TAKING PART IN THE STUDY?

You may or may not receive any benefit from being in this study. It is possible that you may get better, stay the same, or get worse. The risks and benefits of the AMICUS RBCx procedure are believed to be similar to any RBCx procedure. If you take part in this study, other people with sickle cell disease may be helped in the future.

VI. WHAT ARE THE POSSIBLE RISKS OR SIDE EFFECTS (BAD THINGS) OF THE STUDY?

You might have some side effects and discomfort while taking part in this study. The risks to you in this study are similar to the risks related with any RBCx procedure. These risks include a risk of damaged red blood cells, air pocket in the blood system, blood loss, or blood clotting. Although very rare, these risks can cause serious and even life-threatening problems for you like back pain, low blood pressure, different urine color, and lowered kidney function. If your red blood cells are damaged from many procedures, it is possible for you to have increased blood pressure, greater increase in

leg sores, and pressure in your lungs. If you are a man, and your penis is hard for several hours it could harm you (priapism). If air enters your body, it may affect your heart, making it harder for it to pump blood throughout your body. This may make it difficult for your lungs and other organs to receive the right amount of blood flow. If this happens it could damage those organs, or cause pain in your chest. It may also make you have shortness of breath and low blood pressure. In the rare case of that the procedure caused a blood clot, the blood clot may cause pain, swelling, and redness. It can also cause a stroke or be life-threatening. These risks are rare and your doctors and nurses will be watching your vital signs and lab work to reduce the risks if they do occur. Other risks and discomforts that may happen are low blood pressure or blood volume, cooling by the saline or replacement fluids may cause you to feel cold, bruising, allergic reactions, skin redness, itching, hives, trouble breathing rate, stomach cramps, sweating, fainting, tiredness, fast pulse, feeling dizzy, feeling sick to your stomach, and throwing up. Your blood count may go up or down.

During the procedure, you will receive some saline and anticoagulant (also called ACD) that is used to keep your blood from clotting. This is a common anticoagulant solution and is often used other RBCx procedures. An increased risk of bleeding with ACD is very low since your body quickly processes the ACD and the ACD keeps your blood from clotting in the machine.

You may have symptoms of low calcium, such as a tingling feeling, around your mouth, fingers, or toes. You may also have feelings of vibrations, unusual taste, or brief muscle discomfort. You will be closely monitored during the procedure. If these symptoms happen, it is important for you to tell the operator. The operator can then slow the return rate of the blood to reduce the symptoms. If you do not tell the operator the symptoms you have, more serious reactions like muscle twitching or convulsions can happen.

Although donors are tested for numerous diseases, transfusion of blood can spread harmful diseases.

If you are using a medicine called an ACE inhibitor and do not follow the doctor’s instruction to stop taking the medicine before your procedure, you may experience a reddened face, low blood pressure, or increased anxiety during your RBCx procedure.

Although the kit, needles, and central lines (if appropriate) that are used are sterile and proper technique under sterile conditions will be used to prevent infections, in rare cases, infections or inflammation may happen. Sometimes the central lines become clogged.

The system has been designed to lower the possibility that air will enter the kit. A special air detector and bubble trap are present. The air detector is designed to stop the device right away if air enters the kit and the clamps will close, keeping you away from the device. The bubble trap helps to catch air bubbles to keep air from being returned to you. Other sensors stop the device if there is difficulty in removing blood or returning blood. The sensors will stop the device if the kit becomes blocked.

Previous testing of the AMICUS RBCx procedures has been done in the lab.

Testing has shown that the device can reach target fluid balance. But there is a possibility that not enough or too much fluid may be given to you during the procedure. If you do not receive enough fluid, you may experience lightheadedness, temporary loss of consciousness, weakness, thirst, or low blood pressure. If you receive too much fluid and do not have serious heart, lung, or kidney problems, you would be able to get rid of the excess fluid through your urine. However, if you have serious heart, lung, or kidney problems your body may not be able to get rid of the extra fluid. If you cannot get rid of the extra fluid, it is possible that the fluid may enter your lungs and make it difficult for you to breathe. These risks may be greater for you because you have sickle cell disease which affects your blood's ability to carry oxygen throughout your body. The doctor and nurses will monitor the amount of fluid you have throughout the procedure. They will also be sure that you do not have heart, lung, or kidney problems that may increase your risk for these problems.

Due to technical reasons, such as your blood clotting in the device or the presence of a leak in the tubing, it may not be possible to return all of the red blood cells in the device. The maximum amount of red blood cells you would lose if this were to happen would be less than half the volume (about ½ pint) compared to a whole blood donation (about 1 pint).

The AMICUS Exchange Kits that will be used for the study are the same as the FDA approved and commercially available kits used for therapeutic plasma exchange, a procedure that takes out some plasma and replaces it with other similar fluid. The

saline, ACD and replacement fluid containers use color-coded spikes to help the medical staff attach the solutions correctly.

The RBCx procedure may involve unforeseeable risks to you or to your embryo or fetus should you become pregnant prior to taking part in this study. If you find out you are pregnant during the study and up to 30 days after the procedure is completed, you have to tell the study doctor.

VII. WHAT OTHER OPTIONS ARE THERE?

An alternate option would be for you not to take part in this study. You could continue your RBCx treatments using a commercially available device to treat your condition.

VIII. VOLUNTARY PARTICIPATION/TERMINATION (STOPPING) OF RESEARCH

Your participation in this study is voluntary. You are free to choose whether or not to take part in this study. If you do choose to be in the study, you may choose to stop participating at any time by telling the investigator. If you stop participating during an AMICUS procedure, it may not be possible to return all of the red blood cells in the device.

If you stop taking part in this study, you will receive the usual treatment which your doctor would prescribe. There will be no penalty or loss of benefits to which you are otherwise entitled if you choose not to participate. Fresenius Kabi USA, LLC or the Investigator may stop your involvement in this study at any time. For example, this could happen if the doctor feels it would be in your best interest, if you do not follow the rules of the study, or if you are no longer eligible to participate.

IX. WHAT ARE THE COSTS?

You may have to pay for some costs related to this study. These costs may include transportation or parking. The costs for the research RBCx treatment used will be provided to you at no cost. The related study laboratory tests that are not normally done during your treatment will be provided to you at no cost.

X. WILL I BE COMPENSATED FOR MY PARTICIPATION?

You will receive [*$XXX*] to refund study-related costs like transportation and parking. If you do not finish the study, you will be paid for the part of the study that you did complete.

XI. WILL I BE TOLD ABOUT NEW INFORMATION?

If important new information develops during this study that may affect your willingness to keep being in the study, the Investigator, _____ will tell you about this information. If at any time you have questions regarding the research or your participation, you should contact the Investigator, [*telephone#*_____] who will answer all your questions.

If at any time you have questions, comments, or complaints related to how the study is run, or you feel that you have suffered a research-related illness or injury, you may contact the Institutional Review Board/Ethics Commitee at [*telephone#*_____].

A description of this study will be available on http://www.ClinicalTrials.gov, as required by US law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this website at any time.

XII. WHAT DO I DO IF I AM INJURED?

If a bad side effect (also called an adverse reaction) or injury occurs, you will be attended to free of charge by the medical professionals in the treatment area. If necessary, you may be treated by the medical professionals designated by the institution's emergency procedures. However, if you require treatment at an outside hospital or medical center, those costs must be paid by you, your insurance company or other third party payer. If at any time you have questions, comments, or complaints relating to how the study is run, or if you feel you have suffered a research-related illness or injury, you should contact [*Investigator*] at [*telephone#*_____].

XIII. WHO WILL KNOW ABOUT WHAT I DID IN THE STUDY OR HAVE ACCESS TO MY PRIVATE INFORMATION?

The purpose of research studies is to collect medical information from a group of participants in order to determine how well a medical device or treatment works. Therefore, the investigators will need access to the medical records of all of the subjects who take part in the study.

If you sign this consent form, you are giving permission for your physician and [*hospital/care center*] to provide your medical information to the following people, agencies or companies to review and use in this research study:

- Fresenius Kabi USA, LLC

- The United States Food and Drug Administration (FDA)
- Technischer Überwachungs-Verein (Technical Inspection Association, Germany) (TÜV)
- [*List researchers and any other recipients*]_____.

The results of this study will be made available to Fresenius Kabi USA, LLC and will be sent to the FDA and TÜV. Also, your hospital records may be used for data review by Fresenius Kabi USA, LLC, the FDA, and TÜV.

[*Name of the hospital/center*] and your doctors will keep the records of this study confidential, and will release your medical information only to the people or companies listed above. However, you understand that once your doctor or [*name of hospital/center*] releases your medical information to these people or companies, your doctor or [*name of hospital/center*] cannot then ensure that your information will stay confidential. It is possible that these other persons or companies could give your study information to others, without your permission.

The records of this study will be kept confidential with respect to any written or oral reports to the profession or the media. Your name will not be used in the study data, making it impossible to identify you individually.

This signed informed consent form will be placed in your medical record at [*name of hospital/center*] with a copy placed in the Investigator's research file. If you do not have a medical record at [*hospital name/center*], then this signed consent form will be kept in the Investigator's research file.

XIV. WHAT ARE MY RIGHTS AS A PARTICIPANT?

By signing this consent form, you agree to take part in this study. You are not giving up any of your legal rights or releasing [*name of hospital/center*] from responsibility or carelessness.

You may cancel your consent and remove yourself from this study at any time without penalty or loss of benefits. Your treatment by, and relationships with the physician(s) and staff at [*hospital/center*], now and in the future, will not be affected in any way if you do not want to take part or if you enter into the study and then withdraw from it.

At any time, you can tell your doctor or [*hospital/center*] not to use or give out your study information or other information from your medical record to other people or companies. Withdrawal of this permission must be in writing. Any study information or other information from your medical record collected before your written notice of permission withdrawal may still be used for the study, if that information is necessary for the study. Because the purpose of this study is to collect information about the AMICUS Separator, if you refuse to release your study information, you may not be able to start, or continue taking part in this study. Your decision will not affect your regular care and your doctor will not change his or her feelings about you.

If you agree to take part in this research study, you may not be able to look at a copy of your health information collected only for this study while you are taking part in the study. If you wish, you will be able to ask for this study research information when the study is finished. This does not affect your right to see your medical record or the results of tests related to regular medical care that is given during the same time as the research study.

If you have any questions about the research methods, you should contact the Investigator, [*name*], by contacting [*telephone#* _____] during a workday or [*telephone #*_____] at night or on weekends.

You will be given a signed and dated copy of this consent form.

You agree to let your doctor or [*hospital/center*] use and give out your health information in the way it is described in this consent form until the end of the research study.

You have read this consent form, and you agree to take part in this study as explained in this consent form.

Age of Research Subject

Signature of Research Subject (subject is 18 years or older)	Date	Time

Printed Name of Research Subject

I certify that I have explained the above to this research subject and believe that the signature was affixed freely. I also agree to answer any questions that the subject may ask.

Signature of Investigator/Designee Obtaining Consent	Date

Printed Name of Person Obtaining Consent

SAMPLE INFORMED CONSENT FORM

Italicized writing is for guidance only and should not be included in the final document.

Parent Consent to allow Child to Participate in a Research Study

Investigators at *(institution's name)* invite you to consider allowing your child to take part in a research study entitled:

AMIC-003-CMD
Evaluation of the AMICUS Red Blood Cell Exchange (RBCx) System in Sickle Cell Patients

Sponsored by Fresenius Kabi USA, LLC and carried out by *(investigator's name).*

You are being asked to allow your child to be in a research study of a medical device known as the AMICUS Separator.

The purpose of this form is to help you decide if you want to allow your child to be in this study. Please read this form carefully. It may contain words that you do not understand. Please ask the study doctor or nurse to explain any words or information that you do not clearly understand. You may take home an unsigned copy of this form to think about or discuss with family or friends before making a decision.

If you sign this form, you are giving your permission (or consent) for your child to be a part of this study. You can agree to allow your child to take part in this study and change your mind later without any negative effects to you or your child.

You should not sign this form if you have any questions that have not been answered.

I. INTRODUCTION

Blood is made up of four parts: plasma, platelets, white blood cells, and red blood cells. Hemoglobin is a protein found in the red blood cells. It carries oxygen from the lungs to other parts of the body.

You are being asked to allow your child to take part in this study because your child has a hemoglobin disorder called sickle cell disease. Hemoglobin disorders are passed down through families. They are defined by abnormal production and shape of hemoglobin protein. Abnormal hemoglobin production and shape interfere with the

body's ability to circulate blood and carry oxygen throughout the body. This causes a variety of problems and symptoms that your child may have.

Removing red blood cells (which contain abnormal hemoglobin) and replacing them with healthy red blood cells or another fluid is a procedure called red blood cell exchange (also called RBCx). RBCx is used to treat people with some hemoglobin disorders.

II. WHY IS THIS STUDY BEING DONE?

This study is being done because a new procedure, red blood cell exchange (also called RBCx) has been added to the AMICUS Separator.

The AMICUS Separator is a blood collection machine called an apheresis device. It collects blood from the patient. As the blood is collected, the device separates it into its four different parts: plasma, platelets, white blood cells, and red blood cells. The device removes the unwanted blood parts and returns some of the other parts back to the patient along with another fluid, like salt water solution (also called saline), to replace what was removed. This is called the replacement fluid. Replacement fluid is needed to keep the patient's blood level normal.

In RBCx, the red blood cells that are removed from your child are replaced with a new fluid that your child's doctor will prescribe. The doctor may use healthy red blood cells or a mixture of other fluids that do not contain blood (like saline) and blood to replace the red blood cells that were removed.

The parts of the blood that are removed (also called waste blood material) and any samples taken are tested and then discarded after the testing is finished.

The AMICUS Separator can perform similar types of exchange procedures based on the type of replacement fluid the doctor prescribes. This study will look at two types of procedures: RBC Exchange and RBC Depletion/Exchange.

The doctor will prescribe the type of RBCx procedure your child needs based on your child's medical condition.

The AMICUS Separator is approved for treatment procedures. It is also approved for several blood collection procedures in healthy donors. The AMICUS Separator is **not** approved to perform RBCx procedures. Any RBCx procedures performed on the AMICUS Separator are for research purposes only.

This study will look at the AMICUS Separator's RBC Exchange and RBC Depletion/Exchange procedures.

III. SUBJECT SELECTION

Your child can take part in this study if:

- They are 6 years old or older.
- They have sickle cell disease and require RBC Exchange or RBC Depletion/Exchange treatment.
- They are medically stable and have been previously treated with an RBC Exchange or RBC Depletion/Exchange procedure.
- You give informed consent by signing this form before any activities related to this study are started.
- Your child's doctor has enough RBC replacement products that are right for your child's blood type.
- The doctor or nurse can access your child's veins well enough to run the RBCx procedure.
- They are able to tell the doctor or nurse how they are feeling.
- They agree to tell the doctor or nurse any bad side effects they feel during the procedure and about 18-24 hours after the procedure.

Your child cannot take part in this study if:

- The RBCx treatment is done during a hospital emergency visit.
- They were released from the hospital less than a week ago.
- You are not able to fully understand the rules of the study and give informed consent.
- Your child's doctor feels there are factors (such as drug or alcohol abuse, etc.) that could interfere with your child's ability to follow his or her instruction or the rules of the study.
- They have experienced a bad side effect with an RBCx procedure in the past.
- If your child's doctor feels that they have a life expectancy fewer than 30 days.
- You refuse blood products for your child.
- They are pregnant.
- You do not follow the doctor's instructions to stop any of your child's medication that he or she tells you to while taking part in this study.

WHAT IS INVOLVED IN THE STUDY AND HOW LONG WILL I BE IN THE STUDY?

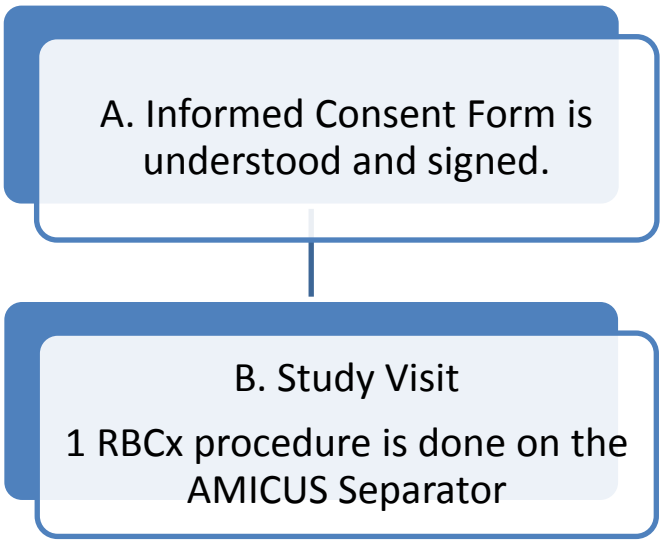
Your child will be 1 of up to 130 subjects taking part in this study at up to 7 study sites.

Your child's doctor will prescribe the type of RBCx procedure that will be used:

- RBC Exchange
- RBC Depletion/Exchange

Your child will be required to complete 1 AMICUS RBCx procedure at 1 Study Visit.

The following figure shows how this study is designed:



RBCx Study Design

During the Study Visit, your child's doctor or nurse will collect information from your child. Your child's date of birth, age at the time this form is signed, sex, ethnicity, race, and the diagnosis of their hemoglobin disorder will be documented. Your child's, height, and weight will be collected. Medicines your child has taken up to 7 days before the Study Visit will be recorded.

Before the start of the procedure, your child's doctor or nurse will measure your child's blood pressure, temperature, pulse, and breathing rate. About 20 milliliters of blood (about 1 ½ tablespoons) will be taken from your child to measure their blood cell counts, test their hemoglobin, hematocrit, and plasma hemoglobin (to determine if red cells have broken). If your child is female with child bearing ability, she will also be required to take a pregnancy test at the beginning of the Study Visit. If she finds out she is pregnant during the study and up to 30 days after the procedure is completed, you have to tell the study doctor.

Two (2) of your child's veins will be accessed using the method your child's doctor or nurse prescribes. In an RBCx procedure, your child's blood will remain within a sterile, single use kit during the procedure. Blood will be taken from one vein and the device will separate it into its 4 parts. Your child's red blood cells will be collected in a plastic bag (waste blood material). Healthy donor red blood cells will be returned to them along with some of their plasma and blood cells in the other vein. Depending on your child's sex, height, weight, and doctor's orders, about 3-5 liters of your child's blood will be processed and returned to them during the RBCx procedure. Only a portion of your child's blood will be removed at one time during the RBCx procedure.

After the procedure is finished, the doctor or nurse will measure your child's blood pressure, temperature, pulse, and breathing rate again. They will also take about 15 milliliters of blood (about 1 ½ tablespoons) to measure your child's blood cell counts, test their hemoglobin, hematocrit, and plasma hemoglobin.

The waste blood material will be tested after the procedure is finished. All samples and waste red blood cells removed will be discarded after testing is finished.

For the RBC Depletion/Exchange procedure, about half way through the procedure, the doctor or nurse will measure your child's blood pressure, temperature, pulse, and breathing rate again. They will also take about 10 milliliters of blood (about 1/2 tablespoon) to measure your child's blood cell counts and test their hemoglobin, hematocrit, and plasma hemoglobin.

If your child's doctor or nurse cannot start the procedure for certain reasons, your child may come back one more time to try again. This may happen if your child's doctor or nurse cannot access their veins or if there isn't enough blood that is right for your child. Your child will be able to come back another time to complete the study procedure.

Your current working telephone number is:

_____(**enter current working phone number and area code**). A member of the study team will call your child about 18-24 hours after the procedure is done to see how they are feeling. The study team will try to call up to 2 times. They will ask your child how they are feeling. Your child will tell them how they are feeling. If the study team is unable to reach you, they will send you a letter in the mail.

An electronic database will be used. Your child's name will not be entered into the database. Your child will be given a unique subject identification number that will be used in place of their name. Your child's date of birth will be entered into the electronic database. The database will be password protected and only approved people will have access to the database.

IV. ARE THERE BENEFITS (GOOD THINGS) TO TAKING PART IN THE STUDY?

Your child may or may not receive any benefit from being in this study. It is possible that they may get better, stay the same, or get worse. The risks and benefits of the AMICUS RBCx procedure are believed to be similar to any RBCx procedure. If your child takes part in this study, other people with sickle cell disease may be helped in the future.

V. WHAT ARE THE POSSIBLE RISKS OR SIDE EFFECTS (BAD THINGS) OF THE STUDY?

Your child might experience some side effects and discomfort while taking part in this study. The risks to your child as a participant in this study are similar to the risks related with any RBCx procedure. These risks include damaged red blood cells, air pocket in the blood system, blood loss, or blood clotting. Although very rare, these risks can cause serious and even life-threatening problems for your child like back pain, low blood pressure, different urine color, and impaired kidney function. If your child's red blood cells are damaged from many procedures, it's possible for your child to have increased blood pressure, greater increase in leg ulcers, and if your child is a boy, priapism. It may also cause your child to experience pressure in their lungs. If air enters their body, it may affect their heart, making it harder for it to pump blood throughout their body. This may make it difficult for their lungs and other organs to receive the right amount of blood flow. If this happens it could damage those organs, or cause pain in their chest.

It may also make your child have shortness of breath and low blood pressure. In the rare case of the procedure causing a blood clot, the blood clot may cause pain, swelling, and redness. It can also cause a stroke or be life-threatening. These risks are rare and your doctors and nurses will be watching your child's vital signs and lab work to reduce the seriousness of these risks if they do occur. Other risks and discomforts that may happen are low blood pressure or blood volume, cooling by the saline or replacement fluids may cause your child to feel cold, bruising, allergic reactions, skin redness, itching, hives, trouble breathing rate, stomach cramps, sweating, fainting, tiredness, fast pulse, feeling dizzy, feeling sick to the stomach, and throwing up. Your child's blood count may go up or down.

During the procedure, your child will receive some saline and anticoagulant (also called ACD) that is used to keep their blood from clotting. This is a common anticoagulant solution and is often used in platelet and white blood cell collection procedures. An increased risk of bleeding with ACD is very low since your child's body quickly processes the ACD and the ACD keeps your child's blood from clotting only in the machine.

Your child may have symptoms of low calcium, such as a tingling feeling, around their mouth, fingers, or toes. They may also have feelings of vibrations, unusual taste, or brief muscle discomfort. Your child will be closely monitored during the procedure. If these symptoms happen, it is important for your child to tell the operator. The operator can then slow the return rate of the blood to reduce the symptoms. If your child does not tell the operator the symptoms they have, more serious reactions like muscle twitching or convulsions can happen.

Although donors are tested for numerous diseases, transfusion of blood has can spread harmful diseases.

If your child is using a medicine called an ACE inhibitor and does not follow the doctor's instruction to stop taking the medicine before their procedure, your child may experience a reddened face, low blood pressure, or increased anxiety during your child's RBCx procedure.

The system has been designed to lower the possibility that air will enter the kit. A special air detector and bubble trap are present. The air detector is designed to stop the device right away if air enters the kit. The clamps will close, keeping your child

away from the device. The bubble trap helps to catch air bubbles to keep air from being returned to your child. Other sensors stop the device if there is difficulty in removing blood or returning blood. The sensors will stop the device if the kit becomes blocked.

Previous testing of the AMICUS RBCx procedures has been done in the lab.

Testing has shown that the device can reach target fluid balance. But there is a possibility that not enough or too much fluid may be given to your child during the procedure. If this happens, it will cause improper fluid balance. If your child does not receive enough fluid, he or she may experience lightheadedness, temporary loss of consciousness, weakness, thirst, or low blood pressure. If they receive too much fluid and do not have serious heart, lung, or kidney problems, they would be able to get rid of the excess fluid through their urine. However, if your child has serious heart, lung, or kidney problems their body may not be able to get rid of the extra fluid. If your child's body cannot get rid of the extra fluid, it is possible that the fluid may enter their lungs and make it difficult for them to breathe. These risks may be greater for your child since they have sickle cell disease which affects the blood's ability to carry oxygen throughout the body. Your child's doctor and nurses will monitor the amount of fluid they have throughout the procedure. They will also be sure that your child does not have heart, lung, or kidney problems that may increase their risk for these problems.

The effects of blood loss are more serious in children than they are in adults. The loss of too much blood may cause your child to have reduced blood flow to their skin and muscle, low blood pressure, weakness, and in serious cases may be life-threatening. Your child's doctors and nurses will be watching your child's vital signs and lab work to reduce the seriousness of these risks if they do occur.

It may be more difficult to access a child's veins for apheresis procedures than for an adult since children are smaller and have smaller veins. Therefore, your child's doctor or nurses may have to use a central line to get venous access in order to use the AMICUS exchange kit. It may be possible, in rare cases, for infections or inflammation to happen. Sometimes the central lines become clogged. Your child's doctor and nurses will use proper sterile technique to help prevent these risks from happening. Your child's doctor will decide the best way to access your child's veins based on their medical need.

Although the kit, needles, and central lines (if appropriate) that are used are sterile and proper sterile technique will be used to prevent infections, in rare cases, infections or inflammation may happen. Due to technical reasons, such as your child's blood clotting in the device or the presence of a leak in the tubing, it may not be possible to return all of the red blood cells in the device. The maximum amount of red blood cells your child would lose if this were to happen would be less than half the volume (about ½ pint) compared to a whole blood donation (about 1 pint).

The AMICUS Exchange Kits that will be used for the study are the same as the FDA approved and commercially available kits used for therapeutic plasma exchange, a procedure that removes some plasma and replaces it with similar fluid. The saline, ACD and replacement fluid containers use color-coded spikes to help attach the solutions correctly.

The RBCx procedure may involve unforeseeable risks to your daughter or to your daughter's embryo or fetus should she become pregnant prior to taking part in this study. If you find out your daughter is pregnant during the study and up to 30 days after the procedure is completed, you have to tell the study doctor.

VI. WHAT OTHER OPTIONS ARE THERE?

An alternate option would be for you to not allow your child to take part in this study. Your child could continue their RBCx treatments using a commercially available device to treat their condition.

VII. VOLUNTARY PARTICIPATION/TERMINATION (STOPPING) OF RESEARCH

Your child's participation in this study is voluntary. You are free to choose whether or not to allow your child to take part in this study. If you do allow your child to be in the study, you may choose to stop their participation at any time by telling the investigator. If you stop your child's participation during an AMICUS procedure, it may not be possible to return all of the red blood cells in the device. If your child stops taking part in this study, your child will receive the usual treatment for their hemoglobin disorder that their doctor would prescribe. There will be no penalty or loss of benefits to which your child is otherwise entitled if you choose to not allow your child to participate. Fresenius Kabi USA, LLC or the Investigator may stop your child's involvement in this study at any time. For example, this could happen if the doctor feels it would be in your child's best

interest, if your child or you do not follow the rules of the study, or if your child is no longer eligible to participate.

VIII. WHAT ARE THE COSTS?

You may have to pay for some costs related to this study. These costs may include transportation or parking. The costs for the research RBCx treatment used will be provided to your child at no cost. The related study laboratory tests that are not normally done during your child's treatment will be provided to your child at no cost.

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You will receive [*$XXX*] per Study Visit to refund study-related costs like transportation and parking. If your child does not finish the study, you will be paid for the part of the study your child did complete.

X. WILL I BE TOLD ABOUT NEW INFORMATION?

If important new information develops during this study that may affect your willingness to allow your child to continue in the study, the Investigator, _____ will tell you about this information. If at any time you have questions regarding the research or your child's participation, you should contact the Investigator, [*telephone#*_____] who will answer all your questions.

If at any time you have questions, comments, or complaints related to how the study is run, or you feel that your child has suffered a research-related illness or injury, you may contact the Institutional Review Board/Ethics Committee at [*telephone#*_____].

A description of this study will be available on http://www.ClinicalTrials.gov, as required by US law. This Web site will not include information that can identify your child. At most, the Web site will include a summary of the results. You can search this website at any time.

XI. WHAT DO I DO IF I AM INJURED?

If a bad side effect (also called an adverse reaction) or injury occurs, your child will be attended to free of charge by the medical professionals in the treatment area. If necessary, your child may be treated by the medical professionals designated by the institution's emergency procedures. However, if your child requires treatment at an outside hospital or medical center, those costs must be paid by you, your insurance company or other third party payer. If at any time you have questions, comments, or

complaints relating to how the study is run, or if you feel you have suffered a research-related illness or injury, you should contact [*Investigator*] at [*telephone#_____*].

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If you sign this consent form, you are giving permission for your child's physician and [*hospital/care center*] to provide your child's medical records to the following people, agencies or companies to review and use in this research study:

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- The United States Food and Drug Administration (FDA)
- Technischer Überwachungs-Verein (Technical Inspection Association, Germany) (TÜV)
- [*List researchers and any other recipients*]_____.

The results of this study may be made available to Fresenius Kabi USA, LLC and will be sent to the FDA and TÜV. Also, your child's hospital records will be used for data review by Fresenius Kabi USA, LLC, the FDA, and TÜV.

[*Name of the hospital/center*] and your child's doctors will keep the records of this study confidential, and will release your child's medical information only to the people or companies listed above. However, you understand that once your child's doctor or [*name of hospital/center*] releases your child's medical information to these people or companies, your child's doctor or [*name of hospital/center*] cannot then ensure that your child's information will stay confidential. It is possible that these other persons or companies could give your child's study information to others, without your permission.

The records of this study will be kept confidential with respect to any written or oral reports to the profession or the media. Your child's name will not be used in the study data, making it impossible to identify them individually.

This signed informed consent form will be placed in your child's medical record at [*name of hospital/center*] with a copy placed in the Investigator's research file. If your child does not have a medical record at [*hospital name/center*], then this signed consent form will only be kept in the Investigator's research file.

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You may cancel your consent and remove your child from this study at any time without penalty or loss of benefits. Your child's treatment by, and relations with the physician(s) and staff at [*hospital/center*], now and in the future, will not be affected in any way if you do not allow your child to take part or if you enter your child into the study and then withdraw them from it.

At any time, you can tell your child's doctor or [*hospital/center*] not to use or give out your child's study information or other information from their medical record to other people or companies. Withdrawal of this permission must be in writing. Any study information or other information from your child's medical record collected before your written notice of permission withdrawal may still be used for the study, if that information is necessary for the study. Because the purpose of this study is to collect information about the AMICUS Separator, if you refuse to release your child's study information, your child may not be able to start, or continue taking part in this study. Your decision will not affect your child's regular care and your child's doctor will not change his or her feelings about them.

If you allow your child to take part in this research study, you may not be able to look at a copy of your child's health information collected only for this study while your child is taking part in the study. If you wish, you will be able to ask for this study research information when the study is finished. This does not affect your right to see your child's medical record or the results of tests related to regular medical care that is given during the same time as the research study.

If you have any questions about the research methods, you should contact the Investigator, [*name*], by contacting [*telephone#* _____] during a workday or [*telephone #*_____] at night or on weekends.

You will be given a signed and dated copy of this consent form.

You give your permission to let your child's doctor or [*hospital/center*] use and give out your child's health information in the way it is described in this consent form.

By signing, you agree that you have read this consent form and you agree to have your child, _____ [*clearly print the child's name*) take part in this study as explained in this consent form.

_____
Age of Child

_____ _____ _____
Signature of Parent(s) or Legal Representative(s) Date Time

_____
Printed Name of Parent(s) or Legal Representative(s)

_____
Identify the signatory: e.g., Parent, Legal Representative

I certify that I have explained the above to this research subject and believe that the signature was given freely. I also agree to answer any questions that the subject may have.

_____ _____
Signature of Investigator/Designee Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent

SAMPLE INFORMED ASSENT FORM

Italicized writing is for guidance only and should not be included in the final document.

Adolescent's Agreement to Participate in a Research Study

Ages 6-17

I am being asked to be in a research study called:

AMIC-003-CMD Evaluation of the AMICUS Red Blood Cell Exchange (RBCx) System in Sickle Cell Patients

The study is being done by (*investigator's name*) at (*name of institution*).

I. WHY IS THIS STUDY BEING DONE?

You are being told about a research study at (*name of institution*). Research studies help explain how medicines and medical devices work. Research studies are by choice, which means that you only have to participate in the study if you want.

You are being asked to be in this research study because you have sickle cell disease. You are being asked to be in this research study to help researchers learn more about a medical device called the AMICUS Separator's Red Blood Cell Exchange (RBCx) System.

II. WHAT HAPPENS IN THE STUDY AND HOW LONG WILL I BE IN THE STUDY?

If you want to be in the study, this is what will happen:

- You will ask any questions that you have. You would sign this form after all of your questions have been answered.
- Your doctor will check to see if you are able to take part in this study.
- You will go to 1 study visit.
- You will complete 1 red blood cell exchange or 1 red blood cell depletion/exchange procedure at the visit. Your doctor will decide what type of procedure will be done.
- You will have 1 procedure using the AMICUS.
- Your doctor or nurse will ask questions about you such as your birthday, age, if you're a boy or a girl and your family background. They will record your height,

weight, and the type of disease that you have. They will record any medicine you have taken up to 7 days before and during the procedure.

- Your doctor or nurse will measure your blood pressure, temperature, pulse and breathing rate before and after the procedure. If the red blood cell depletion/exchange procedure is used, these measurements will also be done about half way through the procedure.
- You will give a small sample of blood (about 1 ½ tablespoons) to measure your blood counts and to see if any of your red blood cells have broken. They will do this before and after the procedure. If the red blood cell depletion/exchange procedure is used, the blood sample and testing will also be done about half way through the procedure.
- If you are a girl and have had your first period, you will give some urine to see if you are pregnant at the beginning of the visit. If you find out you're pregnant during the study and up to 30 days after the procedure is completed, you have to tell your doctor.
- Your doctor or nurse will use 2 of your veins to run the procedure.
- You understand that your blood will be drawn into a kit. The kit is attached to the device and is made up of tubing and plastic bags. The kit will only be used one time.
- Blood will be taken from one vein and the device will separate it into its 4 parts.
- Your red blood cells will be collected in a plastic bag.
- You will be given healthy donor red blood cells and some of your own blood in the other vein.
- Depending on if you are a boy or girl, height, weight, and doctor's orders, about 6-22 cups of your blood will go through the machine and be given back to you. Only a portion of your blood will be removed at one time.
- The blood that the device takes from you will be tested after the procedure is done. The blood will be thrown away after the testing is done.
- Your doctor or nurse will remove you from the machine as usual when the procedure is done.
- The study staff will call you within a day to see how you are feeling. You will tell them how you are feeling.
- The AMICUS RBCx System is an experimental device. This means that the Food and Drug Administration has not approved it for use in the clinic or hospital.

WHAT ARE THE GOOD THINGS ABOUT THE STUDY?

Although you may not be helped directly from this study, you may help the researchers learn something that could help other children in the future who have sickle cell disease.

III. WHAT ARE THE NOT-SO-GOOD, BAD, OR HARMFUL THINGS THAT COULD HAPPEN TO ME IF I AGREE TO BE IN THIS STUDY?

You might have some side effects and discomfort while in the study. Some side effects are tingling in the lips or fingers; strange smell, feeling, or taste; headache; shaking; feeling cold, and in extremely rare cases, your heart may stop beating. It is unlikely, but you may experience skin redness, itching, hives, difficulty breathing, or cramps. It is very rare, but it is possible that air may enter your blood; you may lose blood, or your blood may clot. These rare risks can be very bad and serious. Anytime you receive blood from a healthy donor; there is a risk of getting a harmful disease. But a lot of testing has been done on the blood to help keep this from happening. You may be given too much fluid. If your heart, lungs and kidneys are healthy your body will be able to get rid of the extra fluid when you go to the bathroom. If your heart, lungs, and kidneys are not healthy, it may be difficult for your body to get rid of the extra fluid. If the extra fluid stays in your body, it may get into your lungs and make it hard for you to breathe. If not enough fluid is given to you, you may feel dizzy. Your doctors and nurses will watch you and how much fluid you have during the procedure to keep these problems from happening. Your doctor and nurses will also make sure that your body is healthy enough for the red blood cell exchange procedure.

Even though the doctors and nurses will use the right care, it is possible for you to get a bruise or an infection where they access your veins.

If you have any of these or other bad feelings you must tell your doctor or nurses right away. Your doctor and nurses will help you and try to help you feel better. The study staff will call you the next day after the procedure to see how you are feeling. You will tell them how you are feeling.

You will be told if your doctor learns new information that may make you change your mind about being in this study.

WHAT OTHER OPTIONS ARE THERE?

You do not have to be in this study if you don't want to. Your doctor will still help you.

If you join the study and then change your mind, it is okay for you to leave this study. Your doctors will not be upset with you and will still try to help you feel better.

IV. What about keeping my information private?

Your doctors and nurses will do everything they can to make sure that your medical information and records are kept private.

Unless it's needed by law, only people from the following groups or organizations can see your study records.

- Fresenius Kabi USA LLC
- The United States Food and Drug Administration (FDA)
- Technischer Überwachungs-Verein (Technical Inspection Association, Germany) (TÜV)
- [*List researchers and any other recipients*]_____.

They are required to keep your personal information private.

V. WILL I RECEIVE ANY PAYMENT OR GIFTS IF I AM IN THIS STUDY?

You will receive [*$XXX*] to refund study-related costs like driving and parking. If you do not finish the study, you will be paid for the part of the study that you did finish.

VI. WHAT IF I HAVE QUESTIONS?

You can ask questions whenever you have them. You can ask your doctor, nurse or other people working with them on the study *(insert investigator name and/or study team contact information).* You can also ask your parents.

Your parents know about the study. You don't have to be in the study if you don't want to.

You can ask *(PI name)* anything about the study. If you are not happy with this study and want to talk with someone else, not the doctor or the people working with the doctor, you may contact the IRB/EC at *(insert IRB/EC contact information).*

You will be given a signed and dated copy of this form.

You have read this assent form, and agree to take part in this study as it is explained in this assent form.

_____	_____	_____
Date	Time	Signature of Child (6-17 years old)
_____		_____
Age of Child		Printed Name of Child

_____	_____
Date	Signature of Person Obtaining Assent
	_____
	Printed Name of Person Obtaining Assent

Appendix D. SUMMARY OF PRIOR NON-CLINICAL STUDIES

Summary of Prior Non-Clinical *In Vitro* Studies

During the development phase of the AMICUS RBCx operating protocol, several internal *in vitro* studies using bagged blood were completed to assess the performance, safety and efficacy of RBCx procedures on the AMICUS separator. This included evaluation of the new RBCx application software with the exchange spool/spool holder, AMICUS Exchange Kit (R4R2339) and Blood Component Filter Set with Vented Spike and Luer Adapter (R4C2170) (where applicable) currently cleared for use in AMICUS TPE procedures (K111702, 3/22/12). Where applicable, it also included evaluation of the new ancillary disposable waste transfer set developed for use with AMICUS RBCx procedures.

Feasibility Evaluation of AMICUS RBC Exchange Procedure, 345-REP-018203
An internal *in vitro* feasibility study was performed to evaluate the performance of an RBC Exchange procedure on the AMICUS separator. Data was collected concurrently on the COBE Spectra RBC Exchange procedure for informational purposes. In this study, manually collected units of whole blood drawn from donors were pooled and processed by both the AMICUS and Spectra devices. Measurements and samples were taken pre-procedure and post-procedure to evaluate RBC removal accuracy, end patient Hct accuracy and fluid balance accuracy.

During this study, ten paired procedures were evaluated using pooled whole blood to represent the patient. The pooled blood was warmed for the same amount of time, and mixed using a similar method prior to beginning the procedure on each instrument. The AMICUS inlet flow rate was adjusted to the limit set by Spectra based on the input total blood volume. The average total blood volume for each procedure was approximately 2500 mL. Additional whole blood was hematocrit adjusted to simulate packed red blood cells as the replacement fluid. Patient and procedure parameters were entered such that the two instruments removed a similar volume of red blood cells, targeted the same end patient Hct, and targeted the same fluid balance.

The RBC removal accuracy was determined by calculating the percentage difference between the total actual volume of RBCs removed at the end of the procedure to the targeted RBCs to remove. The values were 5.7% and 6.5% for AMICUS and Spectra, respectively, well within the study acceptance criteria of 8% or less.

The end patient Hct accuracy was determined by comparing the end patient Hct value at the end of each procedure to the targeted end patient Hct value. The average end Hct error for AMICUS was 1.3 Hct points, and for Spectra it was 0.6 Hct points, well within the study acceptance criteria of 5 points or less. The results showed that when the target end Hct is less than or equal to the starting patient Hct, the AMICUS instrument is able to achieve the desired Hct within 2 Hct points; however, when the target End Hct is greater than the starting patient Hct, the error increases to within 3 Hct points. However, in this version of AMICUS software, the system focused on fluid balance as a primary goal and end Hct as a secondary goal.

The fluid balance accuracy was determined by comparing the difference between the actual fluid volume removed and replaced at the end of the procedure and the targeted fluid volume to be removed and replaced, and then dividing it by the total blood volume of the patient. The actual fluid balance error for the AMICUS procedures was 1.4%, well below the study acceptance criteria of less than 10%. This was comparable to Spectra's fluid balance error of -1.4%.

Overall, the averages met the study requirement for RBC removal accuracy, end patient Hct accuracy and fluid balance accuracy. The average results for all three values were within the acceptable ranges identified in the acceptance criteria, and established the feasibility of performing an RBC Exchange procedure on the AMICUS separator.

The ability to achieve FCR was not evaluated in this study. It was assumed that if RBC removal accuracy, end patient Hct and final fluid balance were similar between AMICUS and Spectra procedures, then FCR value would also be comparable. Following this feasibility study, algorithms were implemented in the AMICUS software to include FCR as an endpoint to be achieved simultaneously with End Hct and Fluid Balance. All three endpoints were evaluated in the development phase of the program.

Evaluation of RBCx Procedures in Meeting Key Endpoints

The following objectives were evaluated and are described in the *in vitro* studies referenced below.

Note: Although the RBC Depletion procedure is not included in this clinical protocol, evaluations of this procedure are included in this section for comprehensive purposes.

Objective	Studies
Confirm that AMICUS RBC Exchange, RBC Depletion/Exchange and RBC Depletion procedures meet established internal requirements for key endpoints: FCR, Hematocrit and Fluid Balance.	Evaluation of RBCx Protocol: Evaluation of Amicus RBC Exchange Procedure, 345-REP-028411 Evaluation of RBCx Protocol: Evaluation of Amicus RBC Depletion/Exchange Procedure, 345-REP-030680 Evaluation of RBCx Protocol: Evaluation of AMICUS RBC Depletion Procedure, 345-REP-030681 Evaluation of Amicus RBCx Procedures: Non-Isovolemic Procedures, 345-REP-031764
Evaluate the generation of hemolysis during AMICUS RBC Depletion and RBC Exchange procedures under established worst case scenarios.	Hemolytic Evaluation of AMICUS RBC Depletion and Exchange Procedures, 345-REP-031106
Perform AMICUS RBC Exchange procedures using sickle cell waste blood obtained from patient procedures to examine flow and pressure characteristics and evaluate hemolysis generation.	Evaluation of AMICUS RBC Exchange Procedure Using Sickle Cell Blood, 345-REP-030759

To evaluate the first objective, four separate *in vitro* protocols were conducted to study a combination of FCR, Hct, and Fluid Balance target endpoints. These studies evaluated the performance of the AMICUS RBCx procedures and concurrently collected data on the COBE Spectra Apheresis System.

To simulate an abnormal hemoglobinopathy condition, each procedure used a common pool of Type A whole blood (WB) units to represent the patient's abnormal cells, and a common pool of Type O, AS-1 red cells as the replacement fluid (RF). Flow cytometry was used to measure the fraction of Type A cells remaining in the patient pool at the end of the procedure. This value was analogous to the FCR at the end of the procedure. Equivalent patient and procedure parameters were entered into the AMICUS and Spectra separators and run under the same

environmental conditions. The units of pooled blood were warmed for the same lengths of time and mixed throughout the procedure. Where applicable, a commercial blood warmer was used in both the AMICUS and Spectra procedures.

The internal system requirement specifications for the four studies were:

- The actual FCR shall be within ± 3 of the targeted FCR, on average.
- The procedure shall achieve the targeted end hematocrit with an accuracy of ± 3, on average.
- The fluid balance error (defined as the difference between the actual fluid removed and replaced and the targeted fluid to be removed and replaced, divided by the total blood volume of the patient) shall be less than 5%, on average.
- The difference between the displayed "current fluid balance" and the patient's real-time fluid balance shall be within 15% of the total blood volume of the patient. (Applicable only to Evaluation of Amicus RBCx Procedures: Non-Isovolemic Procedures, 345-REP-031764)

Each of the four studies are summarized below.

Evaluation of RBCx Protocol: Evaluation of Amicus RBC Exchange Procedure, 345-REP-028411

The purpose of this study was to evaluate the performance of the RBC Exchange procedure on the AMICUS separator, and to concurrently collect data on an RBC Exchange procedure on COBE Spectra for comparison. Measurements and samples were taken pre and post-procedure to evaluate the accuracy of FCR, End Patient Hct and Fluid Balance. Intra-procedure sampling was performed where appropriate.

Table 1: Descriptive Statistics of Fluid Balance, FCR, and Hct

	Fluid Balance		FCR		Hct	
	Amicus	**Spectra**	**Amicus**	**Spectra**	**Amicus**	**Spectra**
N	27	27	27	27	27	27
Average	2.83	9.10	1.65	2.99	0.56	0.98
Standard Deviation	1.95	3.57	1.46	2.16	0.47	1.03
Minimum	0.04	0.11	-4.75	-6.69	-1.00	-1.00
Maximum	8.50	16.85	5.03	6.92	1.00	3.50
95% Upper Bound	3.46	10.27	2.13	3.70	0.71	1.32
p value[1]	0.00	1.00	0.00	0.49	0.00	0.00

Note: FCR and Hct data were represented as the difference of target to actual values for the minimum and maximum. The absolute difference of target to actual values was presented for all other data.
[1] Statistical significance was taken at $p < 0.05$.

Since the p-values of the t-test for AMICUS FCR, Hct, and Fluid Balance, and for Spectra Hct, were less than 0.05, there was enough statistical evidence to conclude with 95% confidence that, for AMICUS, the mean was less than 3 points for FCR and Hct and less than 5% for fluid balance.

The results show that the performance of AMICUS, on average, meets the requirements for FCR, Hct and Fluid Balance accuracy and that AMICUS is capable of meeting performance targets for the RBC Exchange procedure. The Spectra device, on average, did not pass the internal requirements established for Fluid Balance accuracy, but did pass for FCR and Hct accuracy.

Evaluation of RBCx Protocol: Evaluation of Amicus RBC Depletion/Exchange Procedure, 345-REP-030680

This study evaluated the performance of the AMICUS RBC Depletion/Exchange procedure, and concurrently collected data on COBE Spectra's Isovolemic Hemodilution - Red Cell Exchange procedure[1] for comparison. The Spectra procedure was performed through the manual connection of two functions: RBC depletion, followed in quick succession by RBC exchange. Measurements and samples were taken pre and post-procedure to evaluate accuracy of FCR, (post-exchange), patient Hct (post-depletion and post-exchange) and Fluid Balance.

Table 2: Descriptive Statistics of FCR, Hct and Fluid Balance

	FCR		Depletion End Hct		Exchange End Hct		Fluid Balance Accuracy	
	Amicus	Spectra	Amicus	Spectra	Amicus	Spectra	Amicus	Spectra
N	24	24	24	24	24	24	24	24
Average	0.97	2.04	0.39	0.12	0.22	0.77	3.43	4.21
Standard Deviation	0.89	1.29	0.38	0.27	0.31	0.78	1.04	1.88
Minimum	-3.97	-3.92	-0.50	-0.75	-1.00	-1.00	1.14	1.08
Maximum	2.51	5.31	1.00	0.50	0.50	3.00	4.97	9.16
95% Upper Bound	1.28	2.50	0.52	0.27	0.33	1.04	3.79	4.87
p value[1]	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.026

[1] Statistical significance was taken at $p < 0.05$.

Since the p-values of the t-test for AMICUS and Spectra FCR, Hct, and Fluid Balance for were less than 0.05, there was enough statistical evidence to conclude with 95% confidence that the AMICUS mean was less than 3 points for FCR and Hct and less than 5% for Fluid Balance.

The results show that the performance of AMICUS, on average, meets the internal requirements for FCR, End Hct and Fluid Balance. The data collected on Spectra meet the internal requirements as well. The averages for AMICUS are overall less than those of Spectra with the exception of the depletion End Hct. Because some depletion replacement fluid was still present in the replacement fluid drip chambers at the end of depletion, the Hct was slightly higher in the simulated patient than the depletion end target. The depletion end target was met at the very beginning of the exchange portion of the procedure when this remaining depletion fluid was returned to the simulated patient. This short delay in hitting the depletion end hematocrit was accounted for by the system which is evidenced by the more accurate exchange end hematocrit. Both end hematocrit averages, however, are within the acceptance criterion set in the protocol. Overall, the results of the study show that the AMICUS is capable of meeting performance targets of the RBC Depletion/Exchange procedure.

Evaluation of RBCx Protocol: Evaluation of AMICUS RBC Depletion Procedure, 345-REP-030681

The report for the evaluation of the AMICUS RBC Depletion procedure is a compilation of data from two *in vitro* studies conducted for the new RBCx protocol: Evaluation of AMICUS RBC Depletion/Exchange Procedure (345-REP-030680) and Hemolytic Evaluation of AMICUS RBC Depletion and Exchange Procedures (345-REP-031106). In the Depletion/Exchange study, the simulated patient was depleted 5 or 10 Hct points from its starting Hct. In the Hemolysis study, Depletion procedures were performed with a high Hct simulated patient which was depleted 30 Hct points from its starting Hct. The following data was used to evaluate the RBC Depletion procedure: the end Hct data at the end of the depletion phase of the Depletion/Exchange procedure performed on AMICUS and the depletion End Hct and Fluid Balance accuracy data from the AMICUS Hemolysis study.

Table 3: Descriptive Statistics of Hct and Fluid Balance Accuracy

	Depletion/ Exchange Study Hct	Hemolysis Study Hct	Overall Hct	Fluid Balance Accuracy
Hematocrit delta	-10% or -5%	-30%		
Replacement Fluid	PBS[2] or Albumin	Saline		
N	24	9	33	9
Average	0.39	0.69	0.47	2.01
Standard Deviation	0.38	0.39	0.40	0.70
Minimum	-0.50	-1.00	-1.00	1.05
Maximum	1.00	0.00	1.00	3.00
95% Upper Bound	0.52	0.94	0.59	2.44
p value[1]	0.000	0.000	0.000	0.000

Note: Hct data is represented as the difference of target to actual values for the minimum and maximum. The absolute difference of target to actual values was presented for all other data.
[1] Statistical significance was taken at $p < 0.05$.
[2]Phosphate Buffered Saline

Since the P-Values of the t-test for hematocrit is less than 0.05, there was enough statistical evidence to conclude with 95% confidence that the mean was less than 3 for Hct.

The results show that the performance of AMICUS, on average, meets the internal requirements for Hct and Fluid Balance accuracy. The Hct data taken from the Depletion/Exchange study were slightly higher than what the system targeted for the depletion End Hct. This is because some depletion replacement fluid was still present in the replacement fluid drip chambers at the end of

the depletion portion of the procedure. The depletion end target was met at the very beginning of the exchange portion of the procedure when this remaining depletion fluid was returned to the simulated patient. However, the data taken from the Depletion/Exchange study were still within the acceptance criterion set in the protocol. Overall, the results of this study show that the AMICUS is capable of meeting performance targets for the RBC Depletion procedure.

Evaluation of Amicus RBCx Procedures: Non-Isovolemic Procedures, 345-REP-031764
During some RBCx procedures, the operator may choose to increase or decrease the blood volume of the patient by adjusting the target fluid balance. This study evaluated the performance of the AMICUS RBC Exchange procedure with an alteration in patient fluid volume (i.e., non-isovolemic). Data was collected for comparison on COBE Spectra. Measurements and samples were taken pre and post-procedure to evaluate the accuracy of FCR, End Patient Hct and FCR.

Table 4: Descriptive Statistics of FCR, Hct and Fluid Balance

	FCR		Hct		Fluid Balance	
	Amicus	**Spectra**	**Amicus**	**Spectra**	**Amicus**	**Spectra**
N	12	12	12	12	12	12
Avg	1.05	5.27	0.42	1.71	2.51	3.44
SD	0.78	3.65	0.42	1.44	1.36	2.59
Min	-2.16	-5.71	-1.0	-3.0	0.33	0.26
Max	1.10	12.31	0.5	4.0	4.77	8.86
95% Upper Bound	1.46	7.16	0.63	2.62	3.21	4.78
p value[1]	0.000	0.973	0.000	0.005	0.000	0.030
Tolerance Bounds	NA	NA	NA	NA	[-6.64,1.82]	[-14.31,10.03]

Note: FCR and Hct data were represented as the difference of target to actual values for the minimum and maximum. The absolute difference of target to actual values were presented for all other data.
[1] Statistical significance was taken at $p < 0.05$.

Since the p-values of the t-test for AMICUS FCR, Hct, and Fluid Balance, and for Spectra Hct and Fluid Balance, were less than 0.05, there was enough statistical evidence to conclude with 95% confidence that the mean was less than 3 points for FCR and Hct and less than 5% for Fluid Balance.

A minimum of 95% of the procedure types performed will fall within the tolerance bounds listed for Fluid Balance with a confidence level of 95%. Neither the tolerance interval for AMICUS nor Spectra included a value greater than 15.00, so these parameters met the internal requirement.

The results showed that the performance of AMICUS on average, meets the requirements for FCR, End Hct and Fluid Balance accuracy. The data collected on Spectra show that it meets the requirements for End Hct and Fluid Balance, but not for FCR. The errors in End Hct and FCR

for Spectra appeared to be correlated with the type of run, both by target fluid balance and total patient blood volume tested. Overall, both the average errors and the standard deviations of all targets were less for AMICUS than for Spectra.

Hemolytic Evaluation of AMICUS RBC Depletion and Exchange Procedures, 345-REP-031106

The objective of this *in vitro* study was to evaluate the amount of hemolysis generated during AMICUS RBC Depletion and RBC Exchange procedures under foreseeable worst case conditions, including:

- Small diameter needles
- Simulated high hematocrit patient blood
- High hematocrit replacement fluids
- Highest allowable whole blood flow rates
- Elevated replacement fluid flow rates
- Use of in-line replacement fluid filters
- No flow conditions through the centrifuge

The elevated Hct levels increased the viscosity of the blood flowing through the disposable that may increase the shear force to which the RBCs were exposed. Therefore, the study primarily examined shear force as a potential cause of hemolysis.

The following internal requirements were evaluated during this protocol:

- The allowable mean difference in a patient's concentration of hemoglobin is less than 25 mg/dL.
- The maximum hemoglobin load which can be given to a patient (45 kg) and results in none/negligible harm is 900 mg.
- The maximum hemoglobin load which can be given to a pediatric patient (10 kg) and results in none/negligible harm is 200 mg.
- The maximum hemoglobin load which can be given to a pediatric patient (10 kg) that results in minor harm is 400 mg.

Ten *in vitro* RBC Depletion procedures were performed with whole blood units from healthy donors that were pooled, spun and had plasma volume removed to simulate patients with high hematocrits. The whole blood flow rate was maintained at the highest possible rate that did not induce inlet pressure related alarms. The RBC Depletion procedures examined the amount of hemolysis generated by the system. A given red cell only passes once through the disposable from the simulated patient to the waste container. Any plasma hemoglobin created during this process was returned to the simulated patient through the separated plasma. The combination of high flow rates, small diameter needles (18 gauge), and high hematocrit blood created a reasonably foreseeable worst case scenario for shear force during an RBC Depletion procedure.

The change in the Patient Plasma Hemoglobin Concentration was the difference between pre and post-plasma hemoglobin levels.

For each Depletion procedure, patient pre and post-procedure and waste RBC product samples were measured for plasma hemoglobin.

Table 5: Plasma Hemoglobin Concentration in RBC Depletion Procedures (mg/dL)

	Patient Pre	Patient Post	Patient Delta	Waste Product
N	10	10	10	10
Avg	32.530	18.520	-14.010[1]	16.860
SD	10.882	6.039	8.101	5.170
Min	23.900	10.100	-34.40	9.200
Max	59.700	26.700	-5.40	23.700

[1] Does not consider the effect of dilution by the RF saline returned to the simulated patient nor the static condition of a bagged blood system.

Hemoglobin mass calculations were also performed for each procedure. The patient delta is the difference between the pre and post-hemoglobin mass.

The change in the System Total Hemoglobin Mass was calculated for each procedure using the following formula:

(Patient Post + Waste) – Patient Pre = System Delta

Table 6: Total Hemoglobin Mass in RBC Depletion Procedures (mg)

	Patient Pre	Patient Post	Patient Delta	Waste Product	System Delta
N	10	10	10	10	10
Avg	167.30	218.90	51.600	94.600	146.30
SD	77.04	77.95	53.158	34.735	72.89
Min	111.00	106.00	-17.000	44.000	39.00
Max	373.00	359.00	124.000	157.000	255.00

Additionally, ten *in vitro* RBC Exchange procedures were performed with high hematocrit replacement fluids. The return flow rates of both the replacement fluid and the plasma were intentionally elevated through procedure targets (i.e., increased target end hematocrit, increased fluid balance). The elevated flow rates exposed the replacement fluid to potentially higher shear forces and maximized the return of plasma free hemoglobin to the simulated patient. The Exchange procedures examined the amount of hemolysis created by both separation and replacement. A given red cell potentially completed a full circuit through the disposable, passing from the RBC replacement fluid container to the simulated patient and then to the waste container. Any free hemoglobin created during this process may eventually be carried back to the simulated patient through the separated plasma. The combination of high hematocrit replacement fluid, the use of a replacement line blood component filter, and the targeting of elevated return rates created a worst case scenario for shear force during an RBC Exchange procedure.

For each Exchange procedure, patient pre and post-procedure, waste plasma, and RF unit samples were measured for plasma hemoglobin.

Table 7: Plasma Hemoglobin Concentration in RBC Exchange Procedures (mg/dL)

	Patient Pre	Patient Post	Patient Delta	RF	Waste Plasma
N	**10**	**10**	**10**	**10**	**10**
Avg	**3.1100**	**30.270**	**27.1701**	**77.760**	**14.010**
SD	**1.8466**	**9.182**	**8.362**	**33.022**	**3.970**
Min	**0.0000**	**16.500**	**15.100**	**37.100**	**7.900**
Max	**6.0000**	**43.600**	**42.000**	**132.600**	**19.700**

[1] Includes the contribution of plasma hemoglobin concentration from the RF.

Hemoglobin mass calculations were also performed for each procedure. The patient delta is the difference between the pre and post-hemoglobin mass.

The change in the System Total Hemoglobin Mass was calculated for each procedure using the following formula:

(Patient Post + Waste) – (Patient Pre + RF) = System Delta

Table 8: Total Hemoglobin Mass in RBC Exchange Procedures (mg)

	Patient Pre	Patient Post	Patient Delta	RF	Waste	System
N	**10**	**10**	**10**	**10**	**9**	**9**
Avg	**40.000**	**340.30**	**300.30**	**273.10**	**127.67**	**149.44**
SD	**23.405**	**99.73**	**87.12**	**89.34**	**44.40**	**85.89**
Min	**0.000**	**190.00**	**169.00**	**138.00**	**72.00**	**12.00**
Max	**76.000**	**447.00**	**426.00**	**413.00**	**202.00**	**279.00**

The results showed that:

- During RBC Depletion procedures, the mean difference in the simulated patient's hemoglobin concentration (-14.010 mg/dL) was significantly less than 25 mg/dL (**Table 5**).
- During RBC Depletion procedures, the mean increase in the simulated patient's hemoglobin mass (51.600 mg) was significantly less than 200 mg (**Table 6**).
- During RBC Exchange procedures, there was not enough statistical evidence to reject the null hypothesis that the hemolysis generation was greater than 25 mg/dL with 95% confidence. Since the RBC Exchange procedures used red cell replacement fluid that already contained some level of hemolysis, this adds variability to the simulated patient's post-procedure hemoglobin concentration. To observe the hemolysis generated by the AMICUS Separator alone, see 345-REP-030759 (**Table 7**).
- During RBC Exchange procedures, the mean increase in the simulated patient's hemoglobin mass (300.30 mg) was significantly less than 400 mg (**Table 8**).

Since this study simulated the real-use condition of using red cell replacement fluid, it is difficult to discern if the hemolysis generated is due to the system or the replacement fluid. Even if it were assumed that all hemolysis generated was due to the system operating under the established worst-case conditions, this would still only present a minor risk of harm.

Evaluate RBCx System Using Waste Sickle Cell Blood
An *in vitro* protocol was conducted to evaluate the performance of the AMICUS RBCx System, including hemolysis generation, using waste sickle cell blood from actual patient RBC Exchange procedures.

Evaluation of AMICUS RBC Exchange Procedure Using Sickle Cell Blood, 345-REP-030759
The purpose of this study was to evaluate the performance of the AMICUS RBC Exchange procedure when processing waste sickle red blood cells obtained from patients who had undergone an RBC Exchange procedure using a commercially cleared therapeutics apheresis device. Samples of simulated patient plasma were collected prior to, during, and after running the RBC Exchange procedure on the AMICUS instrument. The study evaluated the hemolysis generation of the AMICUS system as well as the functionality of the Amicus RBC Exchange procedure when running sickle red blood cells.

Ten procedures were performed using an 18 GA needle and programming the maximum inlet rate at 120 mL/min. Five procedures were performed using an 18 GA needle and programming the maximum inlet rate at 10 mL/min. Additionally, five procedures were performed using a 21 GA needle and programming a maximum inlet rate of 120 mL/min. Not all procedures were able to process blood at the set maximum inlet rate due to low inlet pressure alarms. In these instances, the maximum inlet rate was reduced until the inlet pressure was within the allowable

limits. This is representative of system behavior in normal use and does not affect the validity of the data gathered during these procedures.

During the RBC Exchange procedure, the waste sickle cell blood was drawn into the AMICUS system while saline was returned as RF. After the blood was separated, the plasma was collected and analyzed for plasma hemoglobin levels.

The following internal requirement was evaluated:

The mean difference (pre to post) in the concentration of hemoglobin shall be less than 25 mg/dL in a donor/patient following a procedure.

The data in the following tables demonstrates the change in plasma hemoglobin level from the pre-procedure sample to the final inline procedure sample for each needle size/flow rate configuration noted above.

Table 9: Plasma Hemoglobin Levels, 120 mL/min Max, 18 GA Needle

Date	Actual Inlet Rate (mL/min)	Pre Sample (mg/dL)	Final Inline Sample (mg/dL)	Change in Pls Hb Levels (Pre to Final Inline Sample, mg/dL)
1/30/2014	100	13.5	18.7	5.2
1/31/2014	90	12	13.7	1.7
2/14/2014	120	19.1	13.8	-5.3
2/19/2014	120	1.8	1.5	-0.3
2/21/2014	120	6.9	7.2	0.3
2/26/2014	120	7	8.1	1.1
3/6/2014	120	8.6	9.5	0.9
3/28/2014	120	12.9	12.6	-0.3
4/4/2014	120	20.7	17.5	-3.2
4/21/2014	120	14.6	14.5	-0.1

Table 10: Plasma Hemoglobin Levels, 10mL/min Max, 18 GA Needle

Date	Actual Inlet Rate (mL/min)	Pre Sample (mg/dL)	Final Inline Sample (mg/dL)	Change in Pls Hb Levels (Pre to Final Inline Sample, mg/dL)
3/19/2014	10	13.2	14.5	1.3
3/26/2014	10	12	10.7	-1.3
4/24/2014	10	17.5	17.9	0.4
5/14/14	10	14.7	15.8	1.1
6/6/14	10	121.4[1]	101.9	-19.5

[1]Retrospectively, it was confirmed that the patient's waste product was hemolyzed prior to *in vitro* testing.

Table 11: Plasma Hemoglobin Levels, 120 mL/min, 21 GA Needle

Date	Actual Inlet Rate (mL/min)	Pre Sample (mg/dL)	Final Inline Sample (mg/dL)	Change in Pls Hb Levels (Pre to Final Inline Sample, mg/dL)
4/16/2014	20	9.8	12.6	2.8
4/18/2014	30	89.7[1]	81.5	-8.2
4/23/2014	20	19.9	17.8	-2.1
6/30/14	20	11.5	11.9	0.4
7/14/14	20	18.4	17.8	-0.6

[1]Retrospectively, it was confirmed that the patient's waste product was hemolyzed prior to *in vitro* testing.

The data shows that the pre samples frequently showed less hemolysis than the source blood. This can be explained by the dilution of the source blood by saline already present in the kit following saline prime. As the saline is cleared from the kit, it has less of an effect on hemoglobin levels. Thus, for evaluation of hemolysis generated by the system, the pre sample was compared to the final sample taken from the plasma line as this will include the least amount of saline; while the post-procedure sample will include the saline that has been pumped into the plasma collection bag.

The results show that when the AMICUS RBCx Exchange procedure is performed with 18 and 21 gauge needles at various flow rates, the difference between pre and post procedure plasma hemoglobin values is less than the internal requirement of 25 mg/dL. Additionally, during the procedures, no significant issues were encountered and the procedures proceeded as expected. From this evaluation, it can be concluded that the AMICUS RBC Exchange procedure does not generate hemolysis when processing sickle cell blood at various flow rates through 18 and 21 gauge needles.

Appendix E. SUMMARY OF PRIOR PRE-CLINICAL STUDIES

Summary of Pre-Clinical *In Vivo* Studies

Donor Room Evaluation of the AMICUS Red Blood Cell Exchange (RBCx) System AMIC-002-CMD

This exploratory study was the first human use evaluation in the product development process for the AMICUS RBCx System. The study performed utilized investigational software that can provide three procedures: RBC Exchange, RBC Depletion/Exchange and RBC Depletion. The study evaluated the RBC Depletion procedure in healthy human adult subjects and collected data on the functional operation of the RBCx System (hardware, software, and disposables) prior to initiation of external studies. The protocol enrolled healthy subjects and therefore did not evaluate RBC Exchange or RBC Depletion/Exchange procedures, due to ethical considerations.

EC/IRB approval of the protocol, including the informed consent form was obtained prior to the start of the study. Subjects provided written informed consent prior to participation in the study.

The *in vivo* study included two phases; the Phase 1 consisted of one-way pass abbreviated procedures that collected a single unit of whole blood, (up to 550 mL without anticoagulant) without return of processed fluids or blood components to the subject. The whole blood was drawn from the subject and processed in the AMICUS centrifuge, the red blood cells were collected in the kit waste containers with the plasma collected in a separate attached sterile transfer pack. The return line was connected to a second attached sterile transfer pack.

Phase 2 of the study consisted of two-way pass procedures that removed a total absolute RBC volume up to a maximum of 420 mL, including samples, from subjects and autologous plasma (along with some anticoagulant) was returned with saline replacement fluid. The AMICUS Separator System is currently cleared for the collection of an RBC product of approximately 235 mL in conjunction with a platelet collection (BK000039). Subjects participating in two-way pass procedures were required to meet the eligibility criteria for 2RBC collections using the nomogram developed for the FDA cleared ALYX Component Collection System double RBC apheresis donations.

Phase 1: One-way Pass Procedure Results

In the one-way pass procedures, a total of 8 subjects, 7 male and one 1 female with an average age of 41.6 ± 15.06 (range: 23-64) participated. The saline used as replacement fluid was diverted to a sterile transfer pack attached to the return line needle. The pre to post-procedure changes in the subjects' blood pressure measurements, pulse and temperature were unremarkable. (Table 1)

Table 1 Summary of Subject Parameters (One-way Pass Procedures)

Parameter	N	Mean (SD)	Median	Min, Max
Weight (lbs) (Pre-Procedure)	8	192.4 (28.64)	190.0	153, 236
Height (in) (Pre-Procedure)	8	69.5 (3.82)	70.0	63, 74
Systolic Blood Pressure (mmHg)				
Pre-Procedure	8	119.5 (11.77)	118.0	103, 140
Post-Procedure	7	120.7 (14.08)	122.0	102, 140
Diastolic Blood Pressure (mmHg)				
Pre-Procedure	8	71.1 (11.27)	68.5	57, 95
Post-Procedure	7	77.7 (12.67)	80.0	60, 98
Pulse (bpm)				
Pre-Procedure	8	69.1 (8.51)	70.0	55, 80
Post-Procedure	7	71.4 (9.64)	68.0	60, 84
Temperature (°F)				
Pre-Procedure	8	97.40 (0.888)	97.30	96.4, 98.4
Post-Procedure	7	97.57 (0.637)	97.40	96.8, 98.2
Fingerstick Hematocrit (%)	8	44.06 (4.011)	43.85	38.4, 50.5

Clinical Study Protocol: AMIC-002-CMD
Note: NA=Not Applicable; UNK=Unknown; ND=Not Done
Table Status: DRAFT; SAS Program: Table 14.3.sas: 25OCT2014:21:56:15
Cross reference: Listing 16.3

The subjects' pre to post-procedure changes in hematology (RBC, WBC, platelet counts and hematocrit) were within expected values and no clinically significant differences were observed during the one-way pass procedures. (Table 2)

Table 2 Summary of Subject Hematology (One-Way Pass Procedures)

Parameter	N	Mean (SD)	Median	Min, Max
White Blood Cell Count (WBC) ($x10^3/\mu L$)				
Pre-Procedure	7	5.61 (1.785)	5.10	4.1, 9.0
Post-Procedure	7	5.39 (1.792)	5.10	3.8, 8.8
Red Blood Cell Count (RBC) ($x10^6/\mu L$)				
Pre-Procedure	7	5.059 (0.5348)	5.000	4.42, 5.87
Post-Procedure	7	4.724 (0.5927)	4.710	4.01, 5.60
Platelet Count ($x10^3/\mu L$)				
Pre-Procedure	7	214.7 (15.01)	212.0	190, 238
Post-Procedure	7	214.9 (14.50)	222.0	190, 228
Hematocrit (%)				
Pre-Procedure	7	42.41 (3.948)	41.80	37.7, 47.9
Post-Procedure	7	39.53 (4.455)	39.80	34.2, 45.6
Plasma Hemoglobin (mg/dL)				
Pre-Procedure	7	7.49 (2.781)	7.10	5.0, 13.1
Post-Procedure	7	7.76 (3.754)	6.80	2.4, 13.2

Clinical Study Protocol: AMIC-002-CMD
Note: NA=Not Applicable; UNK=Unknown; ND=Not Done
Table Status: DRAFT; SAS Program: Table 14.4.sas: 26OCT2014:19:48:58
Cross reference: Listing 16.4

The mean subject pre-procedure plasma hemoglobin value was 7.49 ± 2.781 mg/dL (range: 5.0-13.1 mg/dL). Note, the lower level of sensitivity of the plasma hemoglobin assay was 2.4 mg/mL. One subject (05002) reported two mild, single adverse events (infiltration and hematoma) related to an unsuccessful venipuncture prior to the start of the procedure. The subject was treated, recovered and was released without completing the procedure. There was no relationship to the investigational procedure. No serious or unexpected adverse events occurred during this phase of the study.

The procedures were uneventful and comparable to a whole blood donation with a mean duration time of 11.3 ± 1.80 minutes (range: 10-15 minutes). The programmed AC Ratio, citrate infusion rate and the mean whole blood flow rate were 12:1, 1.25 mg/kg/min, and 64.9 ± 9.26 mL/min, (range: 53-80 mL/min), respectively. An average anticoagulated whole blood volume of 495.7 ± 0.49 mL (range: 495-496 mL) was withdrawn from the subjects and separated into separate containers for the waste RBC material and plasma collected. The mean volume of plasma collected was 192.93 ± 26.621 mLs (range: 158.7-231.7). There was no significant hemolysis in the plasma collected during the procedure, as demonstrated by the low mean plasma hemoglobin concentration, 4.21 ± 2.976 mg/dL (range: 2.4 – 10.4 mg/mL).

It was noted that the Fluid Balance from the procedure results had a mean of 9.7 ± 6.24 mL (range: 0-19 mL) when the expected Fluid Balance was zero. Also, the Saline to Subject was observed to be the same values reported for Fluid Balance. Further investigation determined that a positive fluid balance can occur when the saline administration feature used after venipuncture, is active during the Load Replacement Fluid screen, to allow operators to maintain vein patency. As described in the operator's manual, the needle prime should be stopped using the Stop Needle Prime button. In the study procedures in which a minimal volume of saline delivered was reported, the operator did not press the Stop Needle Prime button on the Procedure Setup screen. Prior to pressing the Begin Depletion button to start the procedure, the operator opened the roller clamps on the inlet and return lines. During this brief period of time, saline was sent to the sterile transfer pack that was connected to the return line and was hanging on the side of the instrument positioned below the subject's arm. The AMICUS instrument appropriately reported this saline weight scale difference and updated the Fluid Balance accordingly. Additional training was provided to the Donor Room staff prior to the start of two-way procedures, to minimize the volume of saline administered following venipuncture.

One cassette pressure limit exceeded alarm was reported (Subject # 05001) and was due to the operator forgetting to open a roller clamp on the plasma transfer set. The roller clamp was opened and the procedure was resumed without further incident.

These abbreviated one-way pass procedures verified that the functionality of the RBCx System when used with a human subject supported the data obtained during system verification testing. The changes observed for the subject parameters pre and post-procedure were not clinically significant. No functional issues were identified or unanticipated adverse device effects were reported during these procedures. A procedural observation was made and was addressed with additional training of the Donor Room Staff. Based on the review of these one-way pass procedures, the data were acceptable and demonstrated subject safety to proceed to two-way pass procedures.

Phase 2: Two-way Pass Procedures

The second phase consisted of two-way pass RBC Depletion procedures that removed a limited volume of RBCs and maintained isovolemia with saline and plasma.

Participants met the Sponsor's Donor Room requirements per Standard Operating Procedure for subject safety guidelines for whole blood donations. In addition, the nomogram used with double RBC donations, i.e., the FDA cleared ALYX System Double RBC apheresis collection (Table 2) was used to further qualify subjects and define the maximum total absolute RBC volume that was removed (including samples) based on the subject gender, height, weight, and hematocrit or hemoglobin. The acceptable End Hematocrit was determined prior to the start of the procedure by using the subject's pre-procedure hematocrit, total blood volume, and by adjusting the End Hematocrit until the Depletion Red Cell Volume parameter did not exceed the maximum RBC volume limit for each subject. During the procedure, whole blood was drawn

from the subject and processed. Up to the equivalent of a double RBC collection including samples (i.e., no more than 420 mL of absolute RBCs) was collected in the kit waste containers. Autologous plasma was returned to the subject during the procedure with some anticoagulant and saline replacement fluid. Reinfusion was not performed during any of the procedures.

Table 2 Nomogram for Participation in Two-way Pass Procedures

Subject Weight (lbs)	Height (inches)	Hematocrit (%)	Hemoglobin (g/dL)	Maximum Total Absolute RBC Volume (mL)
Male				
130-149	≥61	≥ 40	≥ 13.3	360
150-174	≥61	≥ 40	≥ 13.3	400
≥175	≥61	≥ 40	≥ 13.3	420
Female				
150-174	≥65	≥ 40	≥ 13.3	360
≥175	≥65	≥ 40	≥ 13.3	400

A total of 18 subjects, 17 male and one 1 female with an average age of 39.3 ± 10.4 (range: 23-56) were enrolled and provided 15 evaluable procedures. All donors met the required 2RBC eligibility criteria. The pre to post-procedure changes in the subjects' blood pressure measurements, pulse and temperature were unremarkable. (Table 3)

Table 3 Summary of Subject Parameters (Two-way Pass Procedures)

Parameter	N	Mean (SD)	Median	Min, Max
Weight (lbs) (Pre-Procedure)	18	204.4 (28.75)	201.0	160, 270
Height (in) (Pre-Procedure)	18	71.2 (3.21)	72.0	65, 76
Systolic Blood Pressure (mmHg)				
Pre-Procedure	18	125.4 (13.39)	126.0	104, 152
Post-Procedure	18	120.4 (10.39)	119.0	102, 142
Diastolic Blood Pressure (mmHg)				
Pre-Procedure	18	78.7 (8.65)	78.5	65, 92
Post-Procedure	18	77.4 (7.88)	78.0	64, 94
Pulse (bpm)				
Pre-Procedure	18	76.6 (10.46)	78.5	56, 96
Post-Procedure	18	73.8 (8.26)	72.0	60, 92
Temperature (°F)				
Pre-Procedure	18	97.80 (0.803)	97.80	96.4, 99.0
Post-Procedure	18	97.77 (0.676)	97.80	96.4, 98.8
Fingerstick Hematocrit (%)	18	45.29 (2.373)	44.80	41.3, 50.2

Clinical Study Protocol: AMIC-002-CMD
Note: NA=Not Applicable; UNK=Unknown; ND=Not Done
Table Status; FINAL; SAS Program: Table 14.3.sas: 14NOV2014:12:31:18
Cross reference: Listing 16.3

The subjects' pre to post-procedure decreases in hematology (WBC, platelet counts, and hematocrit) were not clinically significant and within expected values during a RBC Depletion procedure. The post-procedure RBC counts and hematocrit decreased as anticipated with a double RBC collection. (Table 4)

Table 4 Summary of Subject Hematology (Two-way Pass Procedures)

Parameter	N[1]	Mean (SD)	Median	Min, Max
White Blood Cell Count (WBC) ($x10^3/\mu L$)				
Pre-Procedure	16	6.12 (1.799)	6.05	3.9, 10.4
Post-Procedure	16	5.16 (1.522)	4.85	3.1, 8.7
Red Blood Cell Count (RBC) ($x10^6/\mu L$)				
Pre-Procedure	16	5.094 (0.2843)	4.980	4.84, 5.84
Post-Procedure	16	4.316 (0.2316)	4.335	3.99, 4.85
Platelet Count ($x10^3/\mu L$)				
Pre-Procedure	16	234.1 (40.24)	228.0	174, 318
Post-Procedure	16	211.1 (36.05)	201.5	153, 309
Hematocrit (%)				
Pre-Procedure	16	44.20 (2.373)	43.40	40.8, 49.0
Post-Procedure	16	37.52 (2.287)	37.65	33.9, 43.2
Plasma Hemoglobin (mg/dL)				
Pre-Procedure	16	8.74 (8.655)	5.95	2.4, 35.4
Post-Procedure	16	7.06 (6.237)	5.40	2.4, 25.4
Difference in Plasma Hemoglobin (mg/dL)*	16	-1.69 (3.424)	-0.05	-10.0, 2.3

Clinical Study Protocol: AMIC-002-CMD
*Calculated Parameter:
Difference in Plasma Hemoglobin=Subject Plasma Hemoglobin Post-Procedure - Subject Plasma Hemoglobin Pre-Procedure
[1]Subject #05009 had a venous access issue and did not start the procedure; Subject #05024 had an infiltration and only Pre-Procedure sample was collected
Note: NA=Not Applicable; UNK=Unknown; ND=Not Done
Table Status: FINAL; SAS Program: Table 14.4.sas: 18NOV2014:9:55:56
Cross reference: Listing 16.4

The mean subject pre-procedure and post-procedure plasma hemoglobin values were 8.74 ± 8.655 mg/dL (range: 2.4-35.4 mg/dL) and 7.06 ± 6.237 (range: 2.4-25.4 mg/dL), respectively. The general decrease in plasma hemoglobin post-procedure is attributed to a dilution effect of the saline replacement fluid. One subject (05019) had elevated plasma hemoglobin levels both pre and post-procedure (35.4 mg/dL and 25.4 mg/dL, respectively). Both samples were retested and the original results were confirmed, although the operator did not note any visible hemolysis. The sample was also reported to have a high level of lipemia. Plasma hemoglobin was measured using a spectrophotometric method and presence of turbidity (lipemia) interferes with the measurement.

One subject (05009) was unable to establish dual venous access. Two subjects (05014, 05024) reported one incident each of a mild, single adverse event (AE) return line infiltration. The subjects were treated, recovered and were released without completing the procedure. A third subject (05017) experienced an infiltration prior to the start of the procedure but was recannulated successfully. The infiltration was treated, and the subject completed the procedure. There was no relationship to the investigational article for any of the AEs. No serious or unexpected adverse events occurred during this phase of the study.

The procedures were uneventful with a mean duration time of 14.9 ± 1.62 minutes (range: 12-17 minutes). The programmed AC Ratio, citrate infusion rate and the mean whole blood flow rate were 12:1, 1.25 mg/kg/min, and 69.6 ± 6.48 mL/min, (range: 59-80 mL/min), respectively. An average anticoagulated whole blood volume of 888.5 ± 87.26 mL (range: 770-1047 mL) was withdrawn from the subjects and processed. The mean target End Hematocrit was 39.3 ± 2.77 (range: 35-44) with a mean target absolute Red Cell Depletion Volume of 345.1 ± 19.71 (range: 315-379 mL). The mean replacement fluid volume of saline and ACD-A returned to the subject were 427.9 ± 48.62 mL (range: 381-584 mL) and 49.1 ± 7.19 mL (range: 38-63 mL), respectively. The Fluid Balance reported was 21.6 ± 11.11 mL (range: 10-47 mL).

The RBC waste material collected was weighed and resulted in a calculated mean volume of 476.4 ± 109.85 mL (range: 404-861 mL). The high value of the range (861 mL) was due to air purge volume and additional initial processing saline volume transferred to the waste containers. The hematocrit of the waste material was an average of 52.98 ± 4.952% (range: 39.4-60.4%). The WBC and platelet count were 7.67 ± 2.102 x 10^3/uL (range: 5.0-12.9 x 10^3/uL) and 263.7 ± 51.56 x 10^3/uL (range: 190-406), respectively.

Table 5 summarizes the AMICUS Estimator values calculated at the start of the procedure based on the subject and procedure input compared to the end of procedure reported values.

Table 5 Summary of Estimated Values and Actual Procedure Parameter Results

Parameter	Estimated Value Pre-procedure Mean (SD), n=15	Value Reported Post-procedure Mean (SD), n=15
Procedure Time (min)	13.5 (1.60)	14.9 (1.62)
AC to Subject (mL)	51.1 (6.46)	49.1 (7.19)
AC Required (mL)	68.1 (6.65)	68.3 (6.90)
Other Replacement Fluid[1] (mL)	414.1 (20.06)	427.9 (48.62)
Fluid Balance (mL)	0.00	21.6 (11.11)[2]

[1] Saline used as Replacement Fluid
[2] Volume due to saline used to keep vein open prior to start of procedure

Table 6 summarizes the End Hematocrit accuracy. The mean difference of actual to target End Hematocrit observed may be attributed to the fingerstick hematocrit used to determine study eligibility as the input for the procedure, while an automated measurement was used for the post-

procedure hematocrit. In addition, visible dilution for samples taken from the return line in the first two procedures was noted despite taking a 10 mL volume to waste. Subsequent procedures used the inlet line for sampling post-procedure.

Table 6 End Hematocrit Accuracy (n=15)		
Mean Target End Hematocrit (%)	**Mean Subject Post-Procedure Hematocrit (%)**	**Mean Difference of Actual to Target End Hematocrit (%)**
39.3 (2.77)	37.52 (3.424)	-1.89 (1.784)

Fourteen procedures reported no alarms. During one procedure (05019) the operator did not fill the replacement fluid drip chamber and did not open the clamp resulting in Check Replacement Fluid and Air Detected alarms. The alarms were acknowledged, the roller clamp was opened, and the procedure was resumed without further incident.

The two-way pass RBC Depletion procedures demonstrated that removal of up to approximately 400 mL of RBCs can be safely completed. The AMICUS estimated pre-procedure parameters were consistent with the values reported at the end of the procedure. The changes observed for the subject parameters pre and post-procedure were as anticipated clinically. No functional issues were identified, unanticipated adverse device effects, or serious adverse events were reported during these two-way procedures. The data generated were acceptable and demonstrated subject safety in two-way pass RBC Depletion procedures.

References

1. Matevosyan K, Anderson C, and Sarode R. Isovolemic Hemodilution-Red Cell Exchange for Prevention of Cerebrovascular Accident in Sickle Cell Anemia: The Standard Operating Procedure. J Clin Aph 2012. DOI: 10.1002/jca.21203.